BIOAVAILABILITY AND BIOMETHYLATION OF ARSENIC IN CONTAMINATED SOILS AND SOLID WASTES

By
ROBIN R. RODRIGUEZ

Bachelor of Science
Soil Science
Utah State University
Logan, Utah
1979

Master of Science
Soil Science
Utah State University
Logan, Utah
1981

Master of Science
Civil Engineering
University of Missouri - Columbia
Columbia, Missouri
1994

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Graduate College of the
Oklahoma State University
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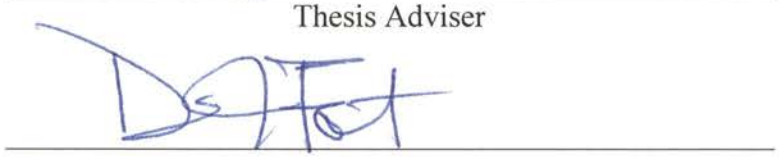
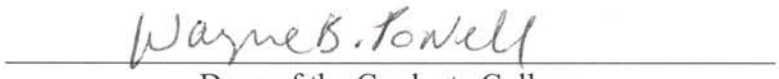
Thesis Adviser





Dean of the Graduate College

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

This dissertation is presented in a series of three chapters. Each chapter is formatted as a stand-alone article following the formatting specifications of *Environmental Science and Technology*. This approach facilitates a more streamlined method of preparing manuscripts for publication of the research without the necessity of re-writing the dissertation.

Data collected from the bioavailability and biomethylation experiments are included in the following appendices:

Appendix I - *In-Vitro* Data

Appenidx II - *In-Vivo* Data

Appendix III - Chemical Speciation Data

Appendix IV - Biomethylation Data

A quality assurance work plan was developed for this project and is included as Appendix V. Data were not collected until all control limits were met.

Chapter 1

An *In-Vitro* Gastro-Intestinal Method to Assess Bioavailable Arsenic in Contaminated Soils and Solid Media

ABSTRACT

A method was developed to simulate the human gastro-intestinal environment to better evaluate the soil ingestion pathway and bioavailability of arsenic as a soil contaminant. The *in-vitro* gastro-intestinal (IVG) method parameters are based upon the medical and nutrition science literature in the concentrations of biochemicals, soil:solution volumes, and experimental conditions. The IVG method is presented with the appropriate conditions representing the two phases of digestion: the stomach phase and the intestinal phase. As an alternative method, and to simulate the adsorption of chemicals across the intestinal membrane, an adsorbent of freshly prepared iron oxide gel was added to the intestinal phase solution (the IVG-AB method). The IVG and IVG-AB method results were compared to *in-vivo* results from the conduct of soil feeding trials using the immature swine model. Results of the IVG stomach phase and intestinal phase were linearly correlated ($r^2 = 0.69$ and 0.67, respectively) with *in-vivo* bioavailable arsenic ($P < 0.01$). The slope of the linear regression lines were 0.88 and 0.76 for the IVG stomach phase and intestinal phase, respectively. This suggests that the IVG method measures nearly as much arsenic as the *in-vivo* method measured. Similar results were demonstrated for the IVG-AB stomach phase and intestinal phase; both phases were linearly correlated ($r^2 = 0.64$ and 0.63, respectively) with *in-vivo* bioavailable arsenic ($P<0.05$). The slopes of the linear regression lines were similar at 0.87

and 0.74 for the IVG-AB stomach phase and intestinal phase, respectively. Analysis of variance showed the IVG stomach phase and intestinal phase, as well as the IVG-AB stomach phase were not statistically different from the *in-vivo* method.

Key Words: arsenic contamination, bioavailability, bioavailable arsenic, incidental soil ingestion, smelter wastes, *in-vitro* method, gastrointestinal simulation, immature swine model

INTRODUCTION

Arsenic is a naturally occurring element typically found in soil at background concentrations ranging from 0.1 to 40 mg kg^{-1} (Bowen, 1979). Arsenic contamination of soil may result from: mining, milling, and smelting of copper, lead, and zinc sulfide ores (Lindau, 1977; Nelson, 1977); raw and spent oil shale (Shendrikar and Faudel, 1978); and coal fly ash (Hansen et al., 1984; Wadge and Hutton, 1987). Arsenic has been found at high levels (10,000 to 20,000 mg kg^{-1}) in some contaminated areas such that the concentration results in unacceptable levels of risk to human health from the incidental ingestion of soil (Life Systems, 1992a, 1992b). Chronic exposure to arsenic may result in skin and internal organ cancers, impaired nerve function, kidney and liver damage, and skin lesions (ATSDR, 1991).

Incidental soil ingestion by children is an important pathway in assessing public health risks associated with exposure to arsenic-contaminated soils. Incidental ingestion of soil results from normal hand-to-mouth activities and represents the principal direct pathway for exposure to non-dietary sources of arsenic in contaminated areas. The importance of soil ingestion by children as a health issue has been reported by numerous researchers and fully illustrates the importance of this pathway in terms of subsequent chemical exposure (Binder

et al., 1986; Calabrese et al., 1989; Clausing et al., 1987; Davis et al., 1990; van Wijnen et al., 1990).

Most risk from arsenic is associated with the forms of arsenic that are biologically available for absorption, or "bioavailable" to humans. A bioavailable chemical is the portion of a chemical dose that enters the systemic circulation from an administered dose. Presently, methods are not available to quantify the amount (as a percentage) of bioavailable arsenic in soils to accurately assess risk from incidental ingestion of arsenic contaminated materials. Hence, some baseline risk assessments developed for contaminated sites have used the conservative assumption that all (i.e. 100%) of the arsenic present in soils and wastes is bioavailable. However, arsenic may exist in many geochemical forms (e.g. oxides, sulfides) and physical forms (e.g. flue dust, slag, tailings, calcine, waste ore) at hazardous waste sites contaminated by mining and smelter wastes. These waste forms vary in their solubilities and geochemical stabilities to the extent that many are not likely to be very bioavailable and therefore may pose only small risks to humans.

The bioavailability of metals, especially lead and arsenic, in some mining wastes have been assessed by conducting expensive and lengthy feeding trials using animal models. The animal model of choice for investigating the enteric bioavailability of arsenic in children requires selection based on similar age and anatomical and physiological characteristics. Pigs are remarkably similar to humans with respect to their digestive tract, nutritional requirements, bone development, and mineral metabolism (Dodds, 1982). Also, pigs, like humans, tend to ingest food intermittently allowing the stomach to evacuate periodically. This physiology is consistent with the way children most likely ingest arsenic-contaminated materials, between

meals when the gastric pH is lowest. Immature pigs have therefore been used successfully as a model for gastrointestinal function of children (Miller and Ullrey, 1987; Weis and LaVelle, 1991).

In order to overcome some of the difficulties and expenses associated with the conduct of animal feeding trials to assess bioavailability of metals in soils, research effort has been directed toward the development of chemical methods which simulate the gastrointestinal environment. One such method is the physiologically-based extracted test (PBET) reported by Ruby et al. (1996). Results for the PBET method have been shown it to be good predictor of lead bioavailability, and the authors reported that their test may have potential for use in determining arsenic bioavailability. However, PBET research with arsenic contaminated materials has been limited to only a small number of materials and these results are inconclusive.

The gastrointestinal digestive processes are quite complicated and difficult to simulate *in-vitro*. Several studies in the area of human nutrition have reported *in-vitro* methods to assess bioavailable iron in foodstuffs (Rao et al.,1978; Miller et al., 1981; Schwartz et al., 1982; and Crews et al., 1983). Many of these procedural steps are based upon the medical and biochemical scientific literature to gain an understanding of the digestive process, especially in terms of digestive solution volumes produced in response to food intake volume, pH conditions during digestive phases, and quantities of digestive juices and enzymes produced such as pepsin, bile acids, pancreatin, etc. (Orten and Neuhaus, 1975; Malagelada et al., 1976).

There are two predominant mechanisms involved during digestion of metals contaminated

soil: the solubility of the metal from the soil matrix and the uptake (absorption) of the metal across the intestinal membrane. Previous *in-vitro* type studies have looked at the solubility of metals under gastrointestinal conditions as an indicator of potential bioavailability (Davis et al., 1992; Ruby et al., 1993; Ruby et al., 1996), but *in-vitro* gastrointestinal methods which simulate the mechanism of absorption have not been reported. Arsenate, and the chemically similar phosphate, have been shown to have a high affinity for amorphous iron oxide gel (Pierce and Moore, 1982; Sharpley, 1991; Myers et al., 1995). Incorporation of iron oxide gel in an *in-vitro* procedure to simulate intestinal absorption is also evaluated in this study.

The primary objective of our study is to develop a method to measure the bioavailable fraction of arsenic in soil and waste which correlates well with the bioavailable arsenic as measured *in-vivo* (per pig feeding trials). A second objective is to compare results from our *in-vitro* studies with those from another *in-vitro* method under development, the PBET (Ruby et al., 1996). An *in-vitro* gastrointestinal technique will provide a rapid, inexpensive testing method to obtain scientifically derived data to select appropriate remedies at contaminated sites which are cost-effective and protective of human health and the environment. A measure of bioavailable arsenic will also serve to lower the uncertainty surrounding the quantification of potential risks arising from exposure to arsenic-contaminated media.

EXPERIMENTAL METHODS

Study Soils and Solid Media

Two matrices were collected for this study from a typical mining/smelter site in the western U.S. where wastes were deposited between 20 and 50 years ago. These aged and

weathered wastes include a calcine material, a waste product which results from the roasting and smelting of arsenopyrite ore for the extraction of arsenic, and an iron slag material, a waste product which results from the smelting of ores for lead which is also high in iron. Five calcine (C1 through C5) and five iron slag (S1 through S5) samples were collected for this from the same site. In addition to the collected soils, five more contaminated solid materials (designated as E1 through E5), which had been archived following previous studies involving chemical analyses and pig feeding trials, were included in the study to test the *in-vitro* method over a broader range of matrices. These materials consist of soils and slags. Chemical and physical properties for the study materials are presented in Table 1 and total arsenic concentrations (ranging from 233 to 17,456 mg kg^{-1}) are presented in Table 2.

Mineralogical composition of one representative calcine (C4) and one representative slag (S4) was determined by microprobe analysis for the various iron and arsenic bearing compounds. The calcine was found to contain: 38% iron-manganese sulfate, 28% iron-arsenic-oxide, and 35% iron-manganese oxide. The iron slag was found to contain: 17% iron-manganese sulfate, 49% iron-arsenic oxide, 4% iron-manganese oxide, 30% lead-manganese oxide, and 2% slag.

Approximately five gallons of each soil was collected, air dried under ambient conditions, and sieved to collect the particle size fraction < 250 μm. This fraction has been determined to be the size which adheres to fingers and is thus available for incidental ingestion. Soils were thoroughly homogenized/mixed prior to use and stored in secured, air-tight containers.

Immature Pig Feeding Trial

Standard operating procedures developed by Dr. Stan Casteel of the University of Missouri-Columbia Veterinary Medicine Diagnostic Laboratory, approved by the U.S. Environmental Protection Agency (EPA) Region 8 (Casteel, 1995), were utilized in the immature pig feeding trials. Intact male pigs weighing 10 to 12 kg were randomly assigned to treatment groups, consisting of: calcine dosing group, slag dosing group, negative control group (no soil), and positive control group (oral $Na_2AsO_4 \bullet 7H_2O$). Five pigs were used per treatment group, with the exception of three pigs per negative control group. All pigs were individually housed in arsenic-free cages and fed low-arsenic feed and water. After a three day acclimation period, the pigs were exposed to soil/treatment doses. Doses were delivered daily (half in morning and half in evening) via 5 g of a vehicle of low-arsenic/low lead diet material (Ziegler Bros., Inc., Gardners, PA), wetted slightly with distilled water to a cookie dough-like consistency. A depression was made in the center, the soil dose was placed into the depression, and the material folded to enclose the dose. The entire vehicle was hand fed to the pigs on schedule. Every three days thereafter, for five collection periods, 24-hour urines were collected from each pig. The urines were filtered (Whatman 2), placed into plastic bottles, and preserved to pH 2 with concentrated HCl. Urine samples were packed securely in coolers on ice and shipped by overnight carrier under chain of custody procedures to Oklahoma State University (OSU) for arsenic analysis. Following an additional filtering through 0.45 μm filters, arsenic analysis was performed by a Thermo-Jarell Ash Inductively Coupled Plasma (ICP) (Maxim) utilizing Hydride Generation (HG). To prepare the urine sample for hydride generation, a 10.0 ml aliquot of urine was placed into a test tube and

mixed with 3.3 ml concentrated HCl and 4.0 ml of a solution containing 10% potassium iodide and 1% ascorbic acid. After a reaction period of at least 1 hour, arsenic was determined by ICP-HG.

In-Vitro Procedures

All *in-vitro* work was conducted in the laboratories of OSU. A conceptual overview of the *in-vitro* study is presented in Figure 1. Bioavailable arsenic was measured in our study by two separate *in-vitro* methods and compared to the *in-vivo* study results. An additional comparison of our *in-vitro* results was made with another previously published *in-vitro* procedure (Ruby et al., 1996; Medlin, 1997). Quart-size canning jars were used as reactor vessels because of their wide-mouth and heavy glass composition. All *in-vitro* procedures were conducted in a water bath at body temperature (37°C), anaerobic conditions were maintained by constantly diffusing argon gas through the solution, and the pH of the *in-vitro* solutions was monitored constantly and adjusted as necessary throughout the procedure. Constant mixing was maintained throughout the procedures (to simulate gastric mixing) by use of individual paddle stirrers at a speed of approximately 100 rpm. A schematic diagram depicting the *in-vitro* reactor design is illustrated in Figure 2.

The *in-vitro* methods were conducted in two separate phases: 1) gastric phase, low pH by adjustment with concentrated HCl (Fisher Scientific, St. Louis, Mo., trace metal grade), followed by 2) intestinal phase, pH raised by adjustment with a saturated solution of $NaHCO_3$. Throughout the gastric and intestinal phases, a small amount of surfactant was added (e.g. decanol) to control excessive foaming due to constant argon gas diffusing through the

solutions. Each phase duration was 1-hour, and at the end of each, a 40 ml sample was collected using a new Luer-lock syringe. Samples were centrifuged at 10,000 rpm for 15 minutes, and the supernatant was filtered through a 0.45 μm filter, acidified to pH 2 with concentrated HCl, and analyzed for arsenic by ICP-HG (following preparation for hydride generation as described above for urine). All *in-vitro* tests were performed in triplicate (simultaneously) for all soils. The three *in-vitro* methods studied are presented below. Adequate blanks, duplicates, and matrix spikes were analyzed to meet quality assurance and quality control requirements. Table 3 presents the experimental parameters for each of the *in-vitro* methods, for clarity, along with their respective literature references.

1) ***In-vitro* Gastrointestinal Method (IVG)**: Gastric phase solution was prepared by making a solution of 0.15 M NaCl (Fisher Scientific, St. Louis, MO.) and 1% porcine pepsin (Sigma Chemical Co., St. Louis, Mo., 146518). Soil (4 g) was added to 600 ml of gastric solution. To mimic the *in-vivo* procedure, an equivalent amount of the dosing vehicle was added to simulate the *in-vivo* dosing technique. Dosing of soil to pigs *in-vivo* was 100 mg soil to 5 g of dough. Therefore, the *in-vitro* reactor included 200 g of dough in the 600 ml of gastric solution to maintain the same ratio (on a volume basis). The pH adjustment of the gastric solution was made following the addition of soil, to account for the various buffering capacities inherent in each soil. The gastric phase solution was modified to the intestinal phase solution by first adjusting the pH to 5.5 with a saturated solution of $NaHCO_3$. Porcine bile extract (Sigma Chemical Co., St. Louis, Mo., Cat. No. B8631) and porcine pancreatin (Cat. No. P1500) were added following pH stabilization, 2.10 and 0.21 g, respectively.

2) In-Vitro **Gastrointestinal Method with Absorption (IVG-AB)**: A second *in-vitro* procedure was performed to determine if an intestinal absorption step could be simulated. The procedure is the same as the IVG method described above with the exception of adding freshly prepared amorphous iron oxide gel during the intestinal phase as an adsorbent. Iron oxide gel is prepared (Myers et al., 1995) by making a 0.65 M $FeCl_3 \cdot 6H_2O$ solution, then slowly adding a solution of 2.7 M NH_4OH until the pH is approximately 6. The amorphous iron oxide gel is then collected by centrifuging the solution at 10,000 rpm for 15 minutes, then carefully pouring off the supernatant. Ten g of iron oxide is placed onto a square (5" x 5") of nylon membrane macroporus filter, 8 μm pore size. Nylon string is then used to tie up the fabric similar to a tea bag which is then allowed to hang freely in the reactor vessel throughout the entire intestinal phase. At the end of the intestinal phase, the iron oxide bag is removed and placed into a 250 ml Erlenmeyer flask. Arsenate is desorbed by adding 200 ml of 0.2 M H_2SO_4 to the flask and shaking on a reciprocal shaker for 1 hour. The resulting solution is filtered through a 0.45 μm pore size filter and analyzed for arsenic by ICP-HG.

3) Physiologically Based Extraction Test (PBET): The PBET procedure was performed as described in Ruby et al., 1996 and Medlin, 1997, with the following exceptions. To maintain anaerobic conditions, argon gas was diffused through the *in-vitro* solutions continuously rather than utilizing closed reactor vessels, and the pH of the gastric solution was raised to 7.0 (to perform the intestinal phase step) by addition of a $NaHCO_3$ solution rather than using dialysis tubing packed with $NaHCO_3$ powder. The $NaHCO_3$ solution reacted much quicker (1.5 to 2 hours faster) and dialysis tubing was subject to breaking by inadvertent contact with the mixing blade.

In-Vivo Bioavailability Calculations

The amount of arsenic which is absorbed through the gastrointestinal tract (bioavailable arsenic) may be described in absolute or relative terms (Casteel, 1995). Absolute bioavailability (ABA) is the ratio of the amount of arsenic absorbed compared to the amount ingested:

$$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose} \qquad \text{Equation (1)}$$

Relative bioavailability (RBA) is the ratio of the absolute bioavailability of arsenic present in some test material (study soil) compared to the absolute bioavailability of arsenic in some appropriate reference material:

$$RBA = \frac{ABA\ (study\ soil)}{ABA\ (reference\ material)} \qquad \text{Equation (2)}$$

In our study, the reference material was the control $Na_2AsO_4 \bullet 7H_2O$ (a readily soluble form, therefore easily absorbed). Arsenic excretion in urine is found to be a linear function of the administered dose (Casteel, 1995), and is approximately independent of time after five days of exposure during feeding trials. Because of the rapid excretion of arsenic via the urine, the urinary excretion fraction (UEF), defined as the amount excreted in the urine divided by the amount dosed, may sometimes be a reasonable approximation of the oral absorption fraction or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile and some enters tissue compartments (e.g. skin,

hair, etc.) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction is not equated with the ABA. The UEF can be used, however, to compute the RBA as follows:

$$RBA\ (of\ soil\ As\ vs.\ reference\ As) = \frac{UEF\ (study\ soil)}{UEF\ (reference\ material)} \qquad \text{Equation (3)}$$

All *in-vivo* bioavailabilities in this study are reported as RBAs.

In-Vitro Bioavailability Calculations

The standard analysis for soil metal content, including arsenic, during the investigation of the nature and extent of contamination of CERCLA sites is by hot digestion with HNO_3 and H_2O_2, USEPA SW 846, Method 3050 (1986). The resulting total metal concentration is then used for estimating risks to human health. The realization that probably not all (100%) of the total metal measured by complete digestion is bioavailable has led risk assessors to use a fraction (percentage) of total metal which better represents the fraction which is bioavailable in the risk calculation. For our *in-vitro* results, bioavailable arsenic is calculated by dividing the arsenic concentration measured in the *in-vitro* stomach phase or the *in-vitro* intestinal phase solutions by that measured as total arsenic (all on a concentration in soil basis), as described by the following equation:

$$In\text{-}vitro\ bioavailable\ As,\ \% = [\frac{in\text{-}vitro\ As,\ mg\ kg^{-1}}{total\ As,\ mg\ kg^{-1}}] * 100 \qquad \text{Equation (4)}$$

For the *in-vitro* method utilizing iron oxide adsorbing gel (IVG-AB), the intestinal phase solution arsenic and the arsenic dissolved from the iron oxide gel are summed to represent the total intestinal phase arsenic.

RESULTS AND DISCUSSION

The length of time to perform the stomach phase and intestinal phase (reaction time) was not clearly described in the literature. Hence, an experiment was conducted using the PBET method (Ruby et al., 1996; Medlin, 1997) (on one calcine and one slag sample) to determine the dissolution of arsenic over time for each of the phases. The soluble concentration of arsenic remained relatively constant during the stomach phase with samples collected every 20 minutes; arsenic concentration results ranged from 1.1 to 1.9 % of total for the calcine and from 13.8 to 15.6% of the total soil arsenic for the slag. Likewise, samples were collected every 60 minutes over a 3-hour intestinal phase and again, arsenic concentration in solution remained relatively constant; arsenic concentration results ranged from 2.0 to 2.3% of total for the calcine and from 10.1 to 10.6% of total for the slag. A 1-hour duration was selected for reaction time of each phase.

Other studies have shown the type of food incorporated into the *in-vitro* method can affect lead bioavailability (Medlin, 1997). In order to replicate conditions of the *in-vivo* experiment as closely as possible, an experiment was conducted to evaluate the condition of food (using the soil dosing vehicle) added versus no food (vehicle) added. An equivalent volume of soil dosing vehicle (which represented 200 g of vehicle) was added to the reactor vessel. One calcine sample and one slag sample were tested with and without food. For the

slag sample, there was no difference in the soluble arsenic measured in either the stomach or intestinal phases of the vessels with food as compared to the vessels without food (21.1 μg g^{-1} stomach phase and 24.4 μg g^{-1} intestinal phase, no-food conditions versus 19.7 $\mu g^{-1}g$ stomach phase and24.3 μg g^{-1} intestinal phase, with-food conditions). However, for the calcine sample, more arsenic was solubilized in the with food treatment as compared to the without food treatment (2.65 μg g^{-1} stomach phase and 6.92 μg g^{-1} intestinal phase, no-food conditions versus 7.86 μg g^{-1} stomach phase and 10.3 μg g^{-1} intestinal phase, with-food conditions). Apparently, adding food would not inhibit arsenic solubilization, and in some cases, may increase arsenic solubilization. For the the IVG and the IVG-AB *in-vitro* experiments, 200 g of dough was added to represent the addition of food.

To simulate absorption across the intestinal membrane, iron oxide gel was added to the IVG-AB intestinal phase solution. To determine the quantity of iron oxide gel to use, an experiment was conducted to determine the effectiveness of various amounts of gel in terms of the amount of arsenic adsorbed from the intestinal phase solution, thereby allowing for potentially more arsenic to become solubilized from the soil. First, iron oxide gel coated filter papers were utilized, prepared as described by Myers et al. (1995). The gel coated filter papers have approximately 80 mg of iron oxide coating on each. Only a small amount of arsenic (approximately 0.1 mg As per g of iron oxide) was adsorbed from a 100 mg L^{-1} intestinal phase solution spiked with sodium arsenate (less than 1% recovery). Greater quantities of iron oxide gel were required to enhance the amount of arsenic adsorbed from solution, and thus, another method was necessary to hold the iron oxide gel in the solution. Experiments were conducted using various mesh sizes of filter fabric to hold 10 g of iron

oxide gel. A mesh size of 8 μm pore size was found to be sufficient in that it allowed water to flow through the fabric, yet did not allow the iron oxide gel to diffuse into the intestinal solution. Using 10 g of freshly prepared iron oxide gel, approximately 1 mg of arsenic was adsorbed per 1 g of iron oxide from a 100 mg L^{-1} spiked intestinal phase solution (16.7% recovery).

The results of all *in-vitro* tests are presented in Figure 3. Although fifteen soils were tested throughout all *in-vitro* experiments, only thirteen xy points are presented on each plot. Two of the slag samples had very low arsenic concentrations and were below *in-vivo* bioavailability detection limits. Results of the IVG stomach phase were linearly correlated (r^2= 0.69) with *in-vivo* bioavailable arsenic ($P<0.01$) (Figure 3a). The slope of 0.88 means the IVG method measures almost the same amount of arsenic as the *in-vivo* method. The IVG intestinal phase was also linearly correlated with *in-vivo* arsenic with a r^2 of 0.67 ($P<0.05$).

Figure 3c presents the results of the IVG-AB stomach phase. Because the IVG-AB stomach phase is the same procedure as for the IVG stomach phase, we would not expect to see much difference between results of Figures 3a and 3c, and in fact, the results are nearly the same. Slight differences were found between the IVG and the IVG-AB intestinal phases (Figures 3b and 3d). Adding the adsorbing material to the *in-vitro* solution decreases the r^2 value of the linear regression line to 0.63 (as compared to 0.67 for IVG), statistically significant at ($P < 0.05$). The slope of the intestinal phase linear regression line of the IVG-AB is nearly the same as the slope of the intestinal phase IVG linear regression line.

The PBET stomach phase results are not linearly correlated with *in-vivo* arsenic, while

the intestinal phase is correlated with an r^2 of 0.57 ($P < 0.05$). The slopes of the PBET regressions (Figures 3e and 3f) were lower than those obtained for the IVG and IVG-AB methods (Figures 3a through 3d). These results suggest the IVG and IVG-AB methods measure more bioavailable arsenic from the contaminated soils than the PBET method.

In consideration the r^2 values of the *in-vitro* tests, it appears that the largest differences are between the PBET test results as compared to the IVG and IVG-AB results. Generally, the PBET method (stomach phase) appears to solubilize approximately half of the arsenic solubilized in the IVG and IVG-AB methods. One important difference between these methods is the amount of pepsin used in the *in-vitro* solutions. The PBET solution contains one-tenth the pepsin concentration of the IVG solutions. We selected the IVG pepsin concentrations from the human nutrition and medical literature (Malagelada et al., 1976; Crews et al.,1983). Pepsin is one of the most important of the digestive enzymes, it hydrolyzes peptide bonds in proteins and polypeptides with a low degree of specificity. Another difference between the two methods is that the PBET method does not incorporate any type of food into the gastric solution. Food has been shown to have an affect on bioavailability (Medlin, 1997).

While correlations represent a method of comparing the *in-vitro* methods with the *in-vivo* method, additional statistical techniques to determine if the *in-vitro* methods are significantly different from the *in-vivo* methods are necessary. An analysis of variance was conducted using the Duncan's Multiple Range Test (SAS, 1997) (Table 4). As shown in Table 4, for all media samples (calcine, iron slag, and miscellaneous soils and slags) only the IVG stomach phase method was equivalent with the *in-vivo* method ($P<0.05$). Evaluating the media

separately, the iron slag material tested by the IVG-stomach phase method was statistically equivalent to the *in-vivo* method , yet the calcine materials were different (at $P<0.05$). When only the slags and soils were evaluated (all media except the calcines), the IVG-stomach phase was still statistically equivalent with the *in-vivo* method at $P<0.05$. Similar results were demonstrated for the IVG-AB-intestinal phase method. When only the slags and soils were evaluated, the IVG-AB intestinal method was statistically equivalent with the *in-vivo* method (at $P<0.05$). The calcine samples, as analyzed by any of the *in-vitro* methods, were not statistically equivalent with the *in-vivo* method. For nearly all groups of materials tested, there are no statistical differences between the IVG stomach phase, IVG intestine phase, and IVG-AB intestine phase. In other words, extending the *in-vitro* method beyond the gastric phase did not improve the ability of the method to measure bioavailable arsenic. Also of note is that neither the PBET stomach phase nor PBET intestinal phase *in-vitro* methods were found to be statistically equivalent with the *in-vivo* method for any groups of contaminated materials tested.

To evaluate and compare the variability of the test results for each of the different methods (*in-vitro* and *in-vivo*), the coefficients of variation (CV) were determined for each (Table 4). The CVs for the IVG and IVG-AB methods for all media were similar; ranging between 73.0 and 75.2%. The IVG and IVG-AB methods were less precise than the *in-vivo* method which demonstrates a CV of 65.6%. The PBET-stomach CV was quite high at 95.2%, while the PBET for the intestinal phase was much lower, and more similar to the IVG methods, at 76.4%. When considering the calcine and slag materials as separate media, the precision results differ greatly. The CVs for the IVG and IVG-AB methods range from 74.0

to 77.2% and the *in-vivo* CV for the calcine media alone is 88.8% (Table 4). The CVs for IVG and IVG-AB methods range from 26.5 to 28.9% and the *in-vivo* CV for the slag media alone is 41.6% (Table 4). When all media is evaluated, except the calcine material, the CV values for all *in-vitro* methods compare well with the CV for the *in-vivo* method. The IVG and IVG-AB method CVs range from 38.7 to 42.4%, while the *in-vivo* CV is 49.1% (Table 4).

It is not expected that any one *in-vitro* method can be developed which will result in an exact replication of *in-vivo* bioavailability. The human digestive system is too complex and dynamic to simulate in the laboratory. A more reasonable approach may be to develop *in-vitro* methods which are based upon human gastro-physiology and correlate well with *in-vivo* method results. From this correlation, mathematical relationships can be developed which will be useful in making risk estimates. The discipline of soil science has used this very concept successfully when early work was performed to find suitable chemical extractants to measure plant available nutrients. Chemical extractants cannot extract plant nutrients in the same manner as a living plant under the conditions of the plant root environment. However, good correlations between soil extractants and plant uptake has allowed soil scientists to use that relationship to make reasonable predictions of plant available nutrients in soil and fertilizer recommendations (Amacher, 1996). Similar relationships between *in-vitro and in-vivo* methods may lead to the development of mathematical relationships from which predictions can be made to derive bioavailable arsenic concentrations in soils for risk estimates which have a lower degree of uncertainty and aid in the design and cost-effectiveness of remedial strategies at contaminated sites.

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Table 1. Major element content and select properties of study media.

Properties	Study Media														
	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5
pH[1]	2.6	2.6	3.1	3.1	5.7	7.4	7.7	7.1	7.4	7.4	3.9	4.6	7.5	7.3	7.6
TOC (%)[2]	0.36	0.22	0.58	0.41	0.61	0.89	3.13	1.58	3.38	3.22	0.81	1.52	2.28	0.23	4.58
% < 2 um[3]	5.7	10.1	13.4	9.8	7.4	6.5	18.4	8.4	7.3	7.5	--	--	--	--	--
% < 50 um[3]	51.6	49.8	57.4	45.5	49.4	30.2	59.1	36.5	45.1	45.6	--	--	--	--	--
Soluble Anions[4]															
	mg kg^{-1}														
Chloride	2545	2950	1237	1079	1711	944	1172	2198	976	1129	4224	9868	912	2446	14171
Sulfate	158281	83119	23368	224253	219776	1308	3287	10509	2898	2529	221126	347176	2484	1360	9449
Nitrate	102.5	159.9	100.3	49.4	297.3	146.9	941.7	507.0	552.2	628.2	323.9	2471.3	53.8	77.5	875.9
Major Elements[5]															
	%														
Si	17.39	17.02	18.00	18.04	22.67	16.83	21.27	23.00	20.05	20.47	22.60	28.83	16.66	12.78	26.54
Al	1.19	1.17	1.60	1.80	3.02	2.43	3.62	3.20	2.64	2.74	7.56	4.64	1.73	2.48	3.56
Ca	1.20	0.68	0.41	2.90	2.86	12.15	9.64	8.57	7.50	6.07	2.43	3.98	12.07	13.99	8.43
Mg	0.16	0.16	0.16	0.22	0.46	1.13	1.53	1.19	1.00	0.95	0.80	0.65	1.33	0.83	1.78
Na	0.14	0.10	0.22	0.24	0.42	0.13	0.33	0.56	0.36	0.36	0.01	0.50	0.01	2.08	0.24
K	0.57	0.53	0.68	0.66	1.15	0.70	1.28	1.01	0.81	0.85	2.99	1.54	0.41	0.66	1.15
Fe	29.66	31.68	28.54	24.97	16.65	20.91	11.68	16.65	17.21	18.33	7.07	2.01	20.37	22.54	6.17
Mn	0.06	0.07	0.06	0.05	0.07	0.21	0.12	0.09	0.12	0.12	1.58	0.28	0.36	0.88	0.11
Ti	0.14	0.14	0.17	0.18	0.29	0.22	0.30	0.25	0.22	0.23	0.40	0.24	0.10	0.22	0.20
Heavy Metals[6]															
	mg kg^{-1}														
Pb	11072	12105	10983	8431	5528	8736	6835	3512	12612	11526	9196	214	11844	12061	3675
Cu	385	318	384	524	997	1807	1606	2208	4228	4009	975	8243	2212	2547	954
Ni	39.1	35.9	31.4	32.2	37.4	24.5	24.4	31.9	35.3	34.0	14.8	17.3	36.7	24.5	24.3

[1]1:1, soil:0.01MCaCl$_2$

[2]Total Organic Carbon (Nelson and Sommers, 1996)

[3]Pipette Method (Gee and Bauder, 1986)

[4]1g soil:10 ml H_2O, Shake 1-hr, Filter 0.45 um

[5]X-Ray Fluor. (Karathanasis and Hajek, 1996)

[6]SW 846, Method 3050 (USEPA, 1986)

Table 2. Chemical content of arsenic in study media.

Arsenic Concentration	Study Media														
	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5
Total (mg kg^{-1})[1]	11294	17456	13472	11525	6245	405	450	1180	5022	4650	331	233	799	1463	401
TCLP (mg L^{-1})[2]	3.0	3.0	3.0	2.9	3.0	3.0	2.8	2.9	3.2	2.9	2.6	2.8	2.6	2.7	2.9
TCLP (mg kg^{-1})[2]	59.4	60.3	60.5	58.8	60.0	59.2	55.6	58.1	63.4	58.6	53.0	55.1	52.2	54.5	59.0
In-Vivo Bioavailable As (%)	2.7	3.3	8.3	22.1	30.1	--	--	28.7	30.1	16.4	6.2	42.8	29.1	18.7	36.5

[1]SW 846, Method 3050 (USEPA, 1986)

[2]SW 846 Method 1311 (USEPA, 1986)

Table 3. *In-vitro* experimental parameters and literature references.

	Method and Reference				
Parameter	IVG :	Reference	IVG-AB :	Reference	PBET (Ruby et al., 1996)
Gastric Solution					
pH	1.8	Malagelada et al., 1976	1.8	Malagelada et al., 1976	2
NaCl	0.15 M	Crews et al., 1983	0.15 M	Crews et al., 1983	none
Pepsin	1.0 %	Crews et al., 1983	1.0 %	Crews et al., 1983	0.10 %
Citrate	none	--	none	--	0.05 %
Malate	none	--	none	--	0.05 %
Lactic Acid	none	--	none	--	0.5 %
Acetic Acid	none	--	none	--	0.50%
Soil:Solution Ratio	1:150	Malagelada et al., 1976	1:150	Malagelada et al., 1976	1:100
Food Added	yes	Casteel, 1995	yes	Casteel, 1995	no
Intestinal Solution					
pH	5.5	Malagelada et al., 1976	5.5	Malagelada et al., 1976	7.0
Pancreatin	0.35 %	Crews et al., 1983	0.35 %	Crews et al., 1983	0.018 %
Bile Extract	0.035 %	Crews et al., 1983	0.035 %	Crews et al., 1983	0.05 %
Adsorbent (Iron Oxide)	no	--	yes	none available	no

Table 4. Analysis of variance using Duncan's Multiple Range Test (SAS, 1997). Values reported are means for that group. Mean values with the same letter designations indicate no difference between groups at $P < 0.05$. Bold values show no differences between *in-vitro* and *in-vivo* methods.

Samples	IVG-Stomach	IVG-Intestinal	IVG-AB-Intestinal	PBET-Stomach	PBET-Intestine	*In-Vivo*	Critical Value*
All Media (n=13)	16.7 ab	14.8 b	15.3 b	11.8 bc	8.26 c	21.0 a	5.3
CV** (%)	75.2	74.6	73.0	95.2	76.4	65.6	
Calcine Only (n=5)	3.66 b	3.52 b	4.00 b	1.44 b	1.47 b	13.5 a	5.1
CV** (%)	74.0	75.9	77.2	77.4	47.3	88.8	
Iron Slag Only (n=3)	24.8 a	22.7 a	24.1 a	13.9 b	12.0 b	25.4 a	7.4
CV** (%)	27.4	28.9	26.6	61.0	33.6	41.6	
All Media Except Calcine (n=8)	24.8 a	21.9 ab	23.0 ab	18.3 bc	12.5 c	25.9 a	6.6
CV** (%)	41.3	42.4	38.7	58.9	40.7	49.1	

*Quanititative difference between means necessary for methods to be significantly different at $P<0.05$.
**CV = Coefficient of variation, %.

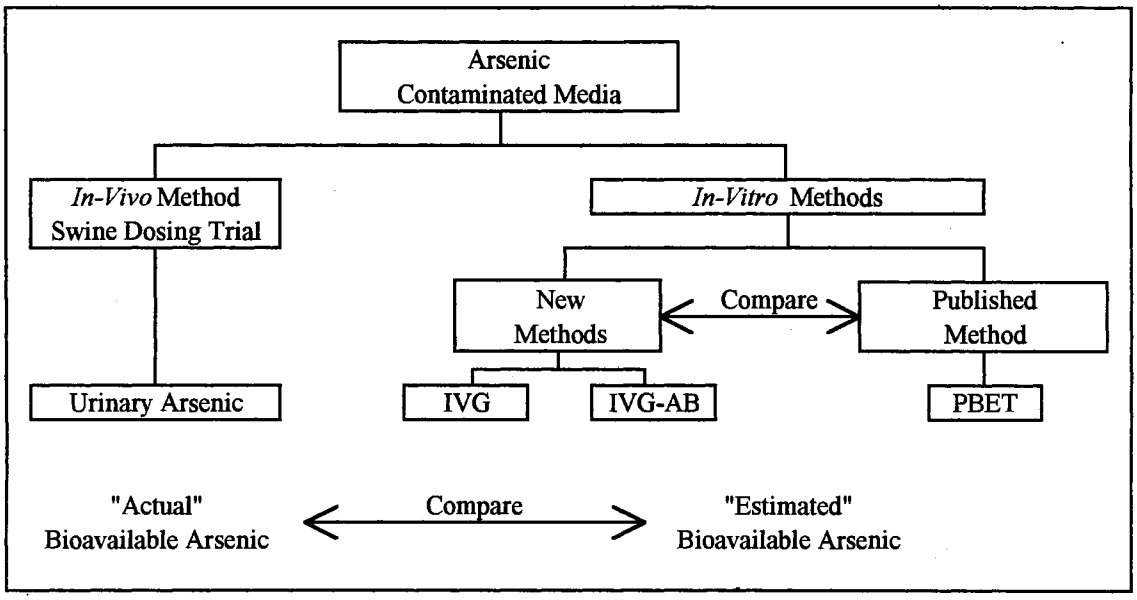

Figure 1. Conceptual approach to the *in-vitro* method evaluation.

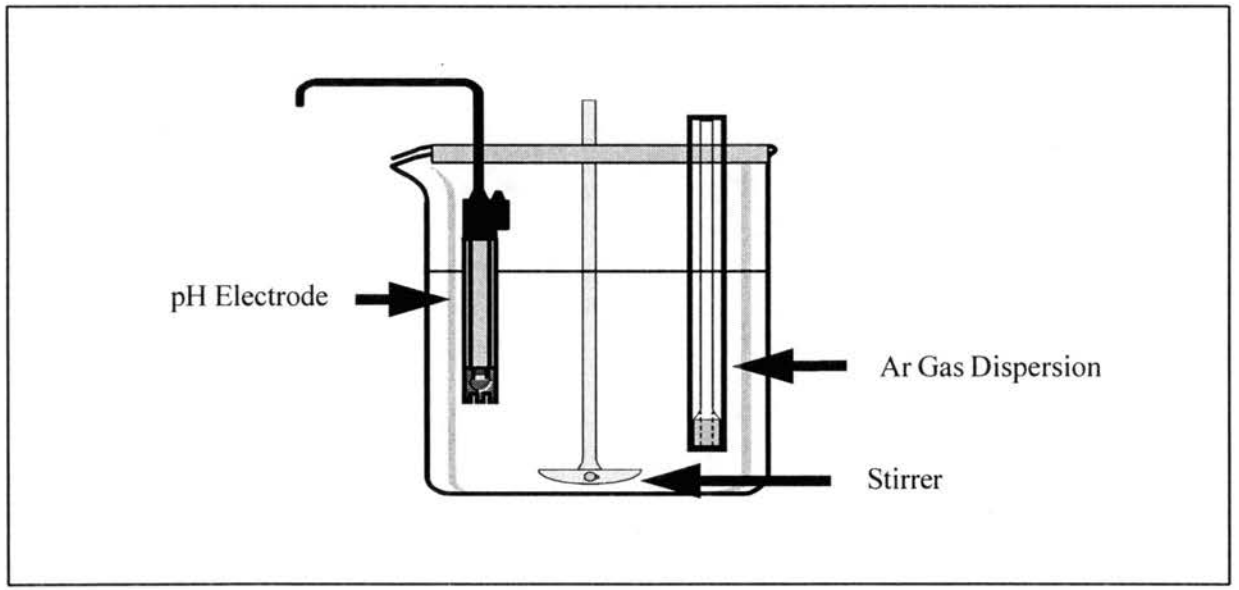

Figure 2. *In-vitro* reactor flask.

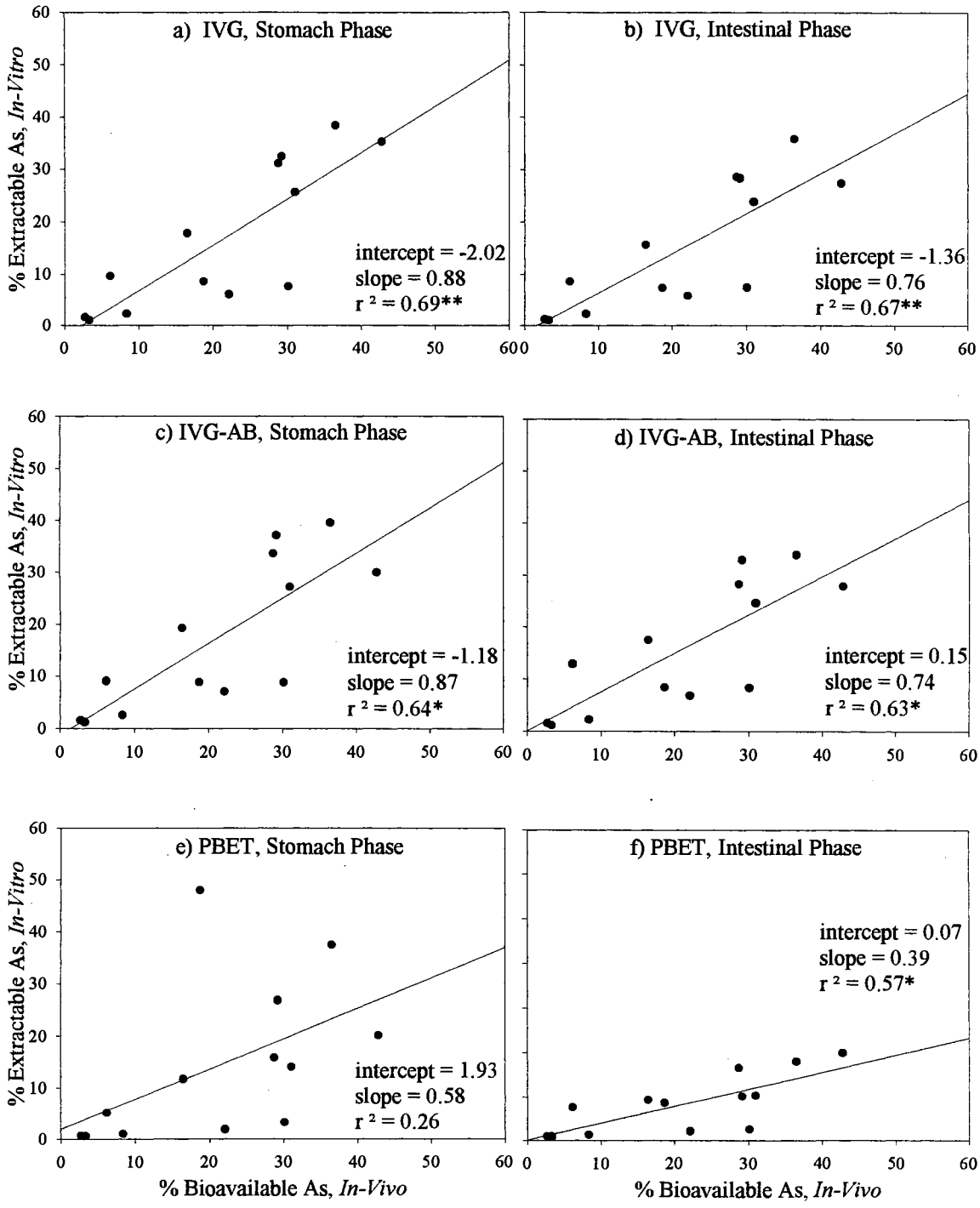

Figure 3. Comparisons of *in-vitro* gastrointestinal stomach and intestinal phase extractable As with *in-vivo* bioavailable As. **Statistically significant, $P < 0.01$; *statistically significant, $P < 0.05$.

Chapter 2

Chemical Speciation Methods to Assess Bioavailable Arsenic in Contaminated Soils and Solid Wastes

ABSTRACT

The soil ingestion pathway has been shown to drive the risk at some hazardous waste sites contaminated with arsenic. Chemical extractants have been described in the literature which extract for various pools of arsenic in soils and solid media, yet none have been evaluated in their ability to measure bioavailable arsenic. A variety of extractants were evaluated in this study and compared to bioavailable arsenic as measured *in-vivo* by animal feeding trials (immature swine model). Extractants such as deionized water, 1M sodium acetate, and 0.1 M Na_2HPO_4/0.1 M NaH_2PO_4 all extract, to varying degrees, the non-occluded or surficial arsenic from soil particles. More aggressive extractants were evaluated which dissolve iron, manganese, and aluminum oxide fractions associated with arsenic in soils. These more aggressive extractants include hydroxylamine hydrochloride, ammonium oxalate, and sodium hydroxide. Individual extractant results were compared with total arsenic, as measured by hot acid digestion (USEPA SW-846, Method 3050), and a chemically extracted percent bioavailable arsenic was obtained. The chemically extracted bioavailability was compared with the *in-vivo* bioavailability to determine the degree of correlation. All extractants were positively correlated. The extractants which measure non-occluded surficial arsenic were found to underestimate the true *in-vivo* bioavailable arsenic, and the more aggressive extractants which dissolve the oxide fractions were found to overestimate *in-vivo* bioavailable

arsenic. The hydroxylamine hydrochloride extractant was the only extractant found to be linearly correlated ($r^2 = 0.54$) with *in-vivo* bioavailable arsenic ($P < 0.10$). The sodium hydroxide extractant was found to come closer to the true value of bioavailable arsenic, yet not close enough to be statistically significant. It appears that the fraction of arsenic in contaminated soils and solid media which is bioavailable is comprised of arsenic fractions between the surficially complexed (desorbable) arsenic and the arsenic associated with the iron/manganese/aluminum oxides.

Key Words: incidental soil ingestion, arsenic contamination, bioavailability, bioavailable arsenic, chemical fractionation, extraction

INTRODUCTION

Arsenic is a naturally occurring element typically found in soil at background concentrations ranging from 0.1 to 40 mg kg^{-1} (Bowen, 1979). Arsenic contamination of soil may result from: mining, milling, and smelting of copper, lead, and zinc sulfide ores (Lindau, 1977; Nelson, 1977); raw and spent oil shale (Shendrikar and Faudel, 1978); and coal fly ash (Turner, 1981; Hansen et al., 1984; Wadge and Hutton, 1987). Arsenic has been found at high levels (10,000 to 20,000 mg kg^{-1}) in some contaminated areas such that the concentration results in unacceptable levels of risk to human health from the incidental ingestion of soil (Life Systems, 1992a, 1992b). Chronic exposure to arsenic may result in skin and internal organ cancers, impaired nerve function, kidney and liver damage, and skin lesions (ATSDR, 1991).

Incidental soil ingestion by children is an important pathway in assessing public health

risks associated with exposure to arsenic-contaminated soils. Incidental ingestion of soil results from normal hand-to-mouth activities and represents the principal direct pathway for exposure to non-dietary sources of arsenic in contaminated areas. The importance of soil ingestion by children as a health issue has been reported by numerous researchers and fully illustrates the importance of this pathway in terms of subsequent chemical exposure (Binder et al., 1986; Calabrese et al., 1989; Clausing et al., 1987; Davis et al., 1990; van Wijnen et al., 1990).

Most risk from arsenic is associated with the forms of arsenic that are biologically available for absorption, or "bioavailable" to humans. A bioavailable chemical is the portion of a chemical dose that enters the systemic circulation from an administered dose. Presently, methods are not available to quantify the amount (as a percentage) of bioavailable arsenic in soils to accurately assess risk from incidental ingestion of arsenic contaminated materials. Hence, some baseline risk assessments developed for contaminated sites have used the conservative assumption that all (ie. 100%) of the arsenic present in soils and wastes is bioavailable. However, arsenic may exist in many geochemical forms (eg. oxides, sulfides) and physical forms (eg. flue dust, slag, tailings, calcine, waste ore) at hazardous waste sites contaminated by mining and smelter wastes. These waste forms vary in their solubilities and geochemical stabilities to the extent that many are not likely to be very bioavailable and therefore may pose only small risks to humans.

The bioavailability of metals, especially lead and arsenic, in some mining wastes have been assessed by conducting expensive and lengthy feeding trials using animal models. The animal model of choice for investigating the enteric bioavailability of arsenic in children requires

selection based on similar age and anatomical and physiological characteristics. Pigs are remarkably similar to humans with respect to their digestive tract, nutritional requirements, bone development, and mineral metabolism (Dodds, 1982). Also, pigs, like humans, tend to ingest food intermittently allowing the stomach to evacuate periodically. This physiology is consistent with the way children most likely ingest arsenic-contaminated materials, between meals when the gastric pH is lowest. Immature pigs have therefore been used successfully as a model for gastrointestinal function of children (Miller and Ullrey, 1987; Weis and LaVelle, 1991).

The method routinely used to characterize arsenic in contaminated solid wastes at hazardous waste sites for remedial investigation and risk assessment purposes is by hot acid extraction using USEPA SW-846, Method 3050 (USEPA, 1986). However, total content may not be related to solubility or bioavailability. Therefore, it is unlikely that total arsenic content will provide an accurate assessment of risk from contaminated materials.

One approach to evaluate solubility and availability of chemical elements in soils involves the use of selective chemical extractants. This approach has been historically used to evaluate plant nutrients in soil. Work performed as early as the 1930's by soil scientists (eg. Bray, Hester, Morgan, Spurway, and Truog) has demonstrated the need to measure "labile" pools rather than total content of nutrients to evaluate conditions for optimum plant growth (Peck and Soltanpour, 1990). Soil test methods for a limited number of metal contaminants have been correlated with plant uptake (Iyengar et al., 1981; LeClaire et al.,1984; Sims, 1986; Soon and Bates, 1982; Xian, 1989), however, little information is available on arsenic.

Chemical speciation methods involving sequential extraction are used to determine the

amount of contaminant in specific chemical pools (Ure, 1990). This approach is dictated by chemical thermodynamics in that the "strongest adsorption sites fill first," which means bioavailability may be a function of contaminant concentration in soil systems. Bioavailability is inversely related to the matrix's ability to adsorb or precipitate the contaminant. Soils have a range of adsorption sites that vary in bonding energies and adsorption strength. Specific adsorption sites in soil strongly retain contaminants rendering them unavailable, whereas weaker adsorption sites will release contaminants making them bioavailable. Most chemical speciation methods have been used to measure heavy metals in ion-exchangeable, surficially adsorbed, precipitated, organic chelated, and occluded chemical pools in baseline soils (Shuman, 1985; Tessier *et al.*, 1979), in sewage sludge-amended soils (Emmerich *et al.*, 1982; Sposito *et al.*, 1982), and in contaminated soils (Gibson and Farmer, 1986; Gupta and Chen, 1975; Hickey and Kittrick, 1984; Kuo *et al.*, 1983; Ma and Rao, 1997; Soon and Bates, 1982). Both heavy metal solubility and bioavailability decrease with each successive step of the sequential extraction.

Because arsenic is chemically similar to phosphorus, it has been evaluated by using chemical extractants developed to measure the various pools of phosphate. Researchers have used this approach to specifically look at various pools of arsenic in soils (Johnson and Barnard, 1979; Gruebel *et al.*, 1988), however they did not correlate the arsenic extracted with plant or biological uptake. A two-step sequential extraction method has been used (Shuman, 1982; Chao and Zhou, 1983) to determine arsenic associated with amorphous iron oxides, manganese oxides, and/or organic matter in wastes, however, an inherent problem associated with this method is the readsorption of arsenic to soil residue after dissolution of

iron oxides. Amacher and Kotuby-Amacher (1994) used phosphoric acid to prevent readsorption of arsenic onto ferrihydrite minerals in a similar chemical speciation procedure. In their extraction, a solution of hydroxylamine hydrochloride and phosphoric acid extracts arsenic associated with amorphous iron and manganese oxides. However, the ability of their method to measure arsenic bioavailability has not been investigated.

The objective of our study is to evaluate the ability of previously reported chemical extractants to measure the bioavailable fraction of arsenic in contaminated soil and solid waste. The chemically extracted arsenic will be evaluated in its correlation with the bioavailable arsenic as measured *in-vivo* (per pig feeding trials). A chemical extractant procedure will provide a rapid, inexpensive testing method to obtain scientifically derived data to select appropriate remedies at contaminated sites which are cost-effective and protective of human health and the environment. A measure of bioavailable arsenic will also serve to lower the uncertainty surrounding the quantification of potential risks arising from exposure to arsenic-contaminated media.

EXPERIMENTAL METHODS

Study Soils and Waste Materials

Two matrices were collected for this study from a typical mining/smelter site in the western U.S. where wastes were deposited between 20 and 50 years ago. These aged and weathered wastes include a calcine material, a waste product which results from the roasting and smelting of arsenopyrite ore for the extraction of arsenic, and an iron slag material, a waste product which results from the smelting of ores for lead which is also high in iron. Five calcine (C1 through C5) and five iron slag (S1 through S5) samples were collected for this study from the same site. In

addition to the collected solid study materials, five more samples of solid materials (designated as E1 through E5) which had been archived following previous studies involving chemical analyses and pig feeding trials were included in the study to test the *in-vitro* method over a broader range of matrices. These materials (E1 - E5) consist of residential soils and slag from smelter areas. Chemical and physical properties are shown in Table 1 and total arsenic concentrations (ranging from 233 to 17,456 mg kg^{-1}) are presented in Table 2.

Mineralogical composition of one calcine (C4) and one slag (S4) was determined by microprobe analysis for the various iron and arsenic bearing compounds. The calcine was found to contain: 38% iron-manganese sulfate, 28% iron-arsenic-oxide, and 35% iron-manganese oxide. The slag was found to contain: 17% iron-manganese sulfate, 49% iron-arsenic oxide, 4% iron-manganese oxide, 30% lead-manganese oxide, and 2% slag.

Approximately five gallons of each study material was collected, air dried under ambient conditions, and sieved to collect the particle size fraction < 250 μm. This fraction has been determined to be the size which adheres to fingers and is thus available for incidental ingestion. Study materials were thoroughly homogenized/mixed prior to use and stored in secured, air-tight containers.

Immature Pig Feeding Trial

Standard operating procedures developed by Dr. Stan Casteel of the University of Missouri-Columbia Veterinary Medicine Diagnostic Laboratory have been approved by U.S. Environmental Protection Agency (EPA) Region 8 (Casteel, 1995), and were utilized in the immature pig feeding trials. Intact male pigs weighing 10 to 12 kg were randomly assigned to treatment groups,

consisting of: calcine dosing group, slag dosing group, negative control group (no soil), and positive control group (oral $Na_2AsO_4 \bullet 7H_2O$). Five pigs were used per treatment group, with the exception of three pigs per negative control group. All pigs were individually housed in arsenic-free cages and fed low-arsenic feed and water. After a three day acclimation period, the pigs were exposed to soil/treatment doses. Doses were delivered daily (half in morning and half in evening) via 5 g of a vehicle of low-arsenic/low-lead diet material (Ziegler Bros., Inc., Gardners, PA), wetted slightly with distilled water to a cookie dough-like consistency. A depression was made in the center, the soil dose was placed into the depression, and the material folded to enclose the dose. The entire vehicle was hand fed to the pigs on schedule. Every three days thereafter, for five collection periods, 24-hour urines were collected from each pig. The urines were filtered (Whatman 2), placed into plastic bottles, and preserved to pH 2 with concentrated HCl. Urine samples were packed securely in coolers on ice and shipped by overnight carrier under chain of custody procedures to Oklahoma State University (OSU) for arsenic analysis. Following an additional filtering through 0.45 μm filters, arsenic analysis was performed by a Thermo-Jarell Ash Inductively Coupled Plasma (ICP) (Maxim) utilizing Hydride Generation (HG). To prepare the urine sample for hydride generation, a 10.0 ml aliquot of urine was placed into a test tube and mixed with 3.3 ml concentrated HCl and 4.0 ml of a solution containing 10% potassium iodide and 1% ascorbic acid. After a reaction period of at least one hour, arsenic was determined by ICP-HG.

In-Vivo Arsenic Bioavailability Calculations

The amount of arsenic which is absorbed through the gastrointestinal tract (bioavailable

arsenic) may be described in absolute or relative terms (Casteel, 1995). Absolute bioavailability (ABA) is the ratio of the amount of arsenic absorbed compared to the amount ingested:

$$ABA = \frac{Absorbed\ Dose}{Ingested\ Dose} \qquad \text{Equation (1)}$$

Relative bioavailability (RBA) is the ratio of the absolute bioavailability of arsenic present in some test material (study soil) compared to the absolute bioavailability of arsenic in some appropriate reference material:

$$RBA = \frac{ABA\ (study\ soil)}{ABA\ (reference\ material)} \qquad \text{Equation (2)}$$

In our study, the reference material was the control $Na_2AsO_4 \bullet 7H_2O$ (a readily soluble form, therefore easily absorbed). Arsenic excretion in urine is found to be a linear function of the administered dose (Casteel, 1995), and is approximately independent of time after five days of exposure during feeding trials. Because of the rapid excretion of arsenic via the urine, the urinary excretion fraction (UEF), defined as the amount excrete in the urine divided by the amount dosed, may sometimes be a reasonable approximation of the oral absorption fraction or ABA. However, this ratio will underestimate total absorption, because some absorbed arsenic is excreted in the feces via the bile and some enters tissue compartments (eg. skin, hair, etc.) from which it is cleared very slowly or not at all. Thus, the urinary excretion fraction is not equated with the ABA. The UEF can be used, however, to compute the RBA as follows:

$$RBA\ (of\ soil\ As\ vs.\ reference\ As) = \frac{UEF\ (study\ soil)}{UEF\ (reference\ material)} \qquad \text{Equation (3)}$$

All *in-vivo* bioavailabilities reported in this report were calculated as RBAs.

Chemical Fractionation Procedures

The various extracting solutions and conditions for chemical fractionation are described below. All samples were extracted in triplicate and included appropriate reagent blanks and spikes. Table 3 presents a summary of the form of arsenic species extracted by the chemical extractants and their respective literature references.

Deionized Water Extraction

One g of soil was placed into a 50 ml polycarbonate centrifuge tube and mixed with 20 ml of deionized water. The tube was closed using a neoprene stopper and placed in a horizontal position on a reciprocal laboratory shaker. The tubes were shaken for one hour and then centrifuged at 8,000 rpm for five minutes. The supernatants were filtered through a 0.45 μm filter using a Luer-lock syringe and acidified to pH 2 with concentrated HCl. Arsenic analysis was performed by ICP-HG (following preparation for hydride generation as described above for urine).

Sodium Acetate Extraction

One g of soil was placed into a 50 ml polycarbonate centrifuge tube and mixed with 20 ml of 1 M sodium acetate solution, pH 5. The tube was closed using a neoprene stopper and placed in a horizontal position on a reciprocal laboratory shaker. The tubes were shaken for one hour and then centrifuged at 8,000 rpm for five minutes. The supernatants were filtered through a 0.45 μm

filter using a Luer-lock syringe and acidified to pH 2 with concentrated HCl. Arsenic analysis was performed by ICP-HG (following preparation for hydride generation as described above for urine).

Phosphate Extraction

One g of soil was placed into a 50 ml polycarbonate centrifuge tube and mixed with 20 ml of a solution consisting of 3 parts of 0.1 M Na_2HPO_4 to 2 parts of 0.1 M NaH_2PO_4 (as described by Yamamoto, 1975). The tube was closed using a neoprene stopper and placed in a horizontal position on a reciprocal laboratory shaker. The tubes were shaken for eight hours and then centrifuged at 8,000 rpm for five minutes. The supernatants were filtered through a 0.45 μm filter using a Luer-lock syringe and acidified to pH 2 with concentrated HCl. Arsenic analysis was performed by ICP.

Hydroxylamine Hydrochloride Extraction

One g of soil was placed into a 250 ml polystyrene centrifuge bottle and mixed with 250 ml of a solution made of the following: 0.25 M NH_2OH, 0.25 M HCl, and 0.025 M H_3PO_4 (as described by Amacher and Kotuby-Amacher, 1994). The centrifuge bottles were placed into a 70°C water bath and shaken for two hours. The bottles were then centrifuged for 10 minutes at 6,000 rpm and the supernatants were filtered through a 0.45 μm filter. Arsenic analysis was performed by ICP.

Ammonium Oxalate Extraction

One g of soil was placed into a 250 ml polystyrene centrifuge bottle and mixed with 50 ml of a solution made of the following: 0.2 M $(NH_4)_2C_2O_4$•HCl, 0.2 M $C_2H_2O_4$•HCl, and 0.025 M H_3PO_4. The centrifuge bottles were placed into a 100°C water bath for 15 minutes. The bottles

were then removed and filtered through 0.45 μm filter. The solid material on the filter was then washed with an additional 50 ml of the fresh extracting solution to yield a final volume of 100 ml. Arsenic analysis was performed by ICP.

Total Arsenic Determinations

Total arsenic was determined by hot acid extraction using USEPA SW-846 Method 3050 (USEPA, 1986).

Chemically Extracted Arsenic Bioavailability Calculations

The standard analysis for soil metal content, including arsenic, during the investigation of the nature and extent of contamination of hazardous waste sites is by hot digestion with HNO_3 and H_2O_2, USEPA SW 846, Method 3050 (1986). The resulting total metal concentration is then used for estimating risks to human health. The realization that probably not all (< 100%) of the total metal measured by complete digestion is bioavailable has led risk assessors to use a fraction (percentage) of total metal which better represents the fraction which is bioavailable in the risk calculation. For our chemically extracted results, bioavailable arsenic is calculated by dividing the arsenic concentration measured by the various chemical speciation extractions by that measured as total arsenic (all on a concentration in soil basis), as described by the following equation:

$$Extracted\ bioavailable\ As,\ \% = \left[\frac{extracted\ As,\ mg\ kg^{-1}}{total\ As,\ mg\ kg^{-1}}\right] * 100 \qquad \text{Equation (4)}$$

RESULTS AND DISCUSSION

The results of the various arsenic chemical extractants, as well as the total arsenic results, are presented in Table 4. Since these extractions were conducted separately, rather than performed sequentially, more than one fraction of arsenic pool was extracted (Table 3). Very little arsenic was extracted by the deionized water, generally less than 5 mg kg^{-1} of water soluble arsenic (Table 4). The sodium acetate extractant, which measures the weakly exchangeable pool of arsenic as well as the water soluble pool, resulted in more arsenic extracted than the water soluble fraction; between 32 and 195 mg kg^{-1} of arsenic. The phosphate solution extracted a greater portion of arsenic; results ranged between 70 and 380 mg kg^{-1} of arsenic. This fraction represents the strongly (specifically) adsorbed fraction of arsenic, or some portion of that fraction, as well as the water soluble and weakly exchangeable fractions. All of these three extractants can best be described as extracting non-occluded, or surface layers of the soil particles.

Hydroxylamine hydrochloride, a much more aggressive extractant that dissolves iron and manganese oxides, extracts surficial adsorbed arsenic and some of the occluded arsenic in the mineral matrix. Substantially more arsenic is measured by extraction with hydroxylamine hydrochloride as compared to the extractions by water, sodium acetate, and phosphate; results ranged between 186 and 4,830 mg kg^{-1} (Table 4). As proposed by Chao and Zhou (1983), hydroxylamine hydrochloride extracts arsenic which is associated with amorphous manganese and iron oxides. As proposed by Shuman (1982), ammonium oxalate, which is more aggressive than hydroxylamine hydrochloride, extracts arsenic associated with both the amorphous and crystalline iron oxides, as well as the aluminum oxide fraction. Ammonium oxalate extracted between 408 and 12,616 mg kg^{-1} of arsenic (Table 4). To release arsenic from a crystalline matrix requires a

more thorough dissolution of the soil particle. The sodium hydroxide extractant released an intermediate amount of arsenic into solution; between 975 and 9,092 mg kg^{-1} of arsenic (Table 4). The sodium hydroxide extractant should measure arsenic associated with the aluminum oxide fraction (Olsen and Sommers, 1982). The sodium hydroxide procedure dissolves the aluminum oxide surface and some of the mineral fraction.

To compare the chemically extracted arsenic results with arsenic bioavailability, the extractant results were expressed as a percentage of total content (Table 5). The trend of arsenic extracted bioavailability is as expected, the more aggressive extractants result in higher chemically measured bioavailabilities. Some extractants approach and even exceed 100% of total arsenic (measured by USEPA SW-846, Method 3050), such as the ammonium oxalate extractant of some of the study materials. The relationship between chemically extracted arsenic and actual *in-vivo* bioavailable arsenic was determined (Figure 1). The plots are prepared in xy pairs of data where x represents the % bioavailable arsenic as measured in the *in-vivo* study and the matching y value represents the %extracted arsenic as measured in the corresponding chemical extractant. Although fifteen soils were tested throughout all chemical extracting experiments, only thirteen xy points are presented on each plot. Two of the slag samples had very low arsenic concentrations and were below detection limits of the *in-vivo* method. All of the chemical extractants produced a positive linear regression line, however, only one extractant, hydroxylamine hydrochloride, was linearly correlated ($r^2 = 0.54$) with *in-vivo* bioavailable arsenic ($P < 0.10$). However, the chemically extracted bioavailability for this extractant over-estimated the true bioavailability as evident by the high y-intercept (24.4) and slope of the linear regression line greater than one (1.45).

Further statistical analysis was performed to determine if there were significant differences

between the means of the chemically extracted arsenic methods. An analysis of variance was conducted using Duncan's Multiple Range Test (SAS, 1997) (Table 6). For all media tested (calcine, slags, and soils) the sodium acetate and sodium hydroxide extractable arsenic were not significantly different from the *in-vivo* bioavailable arsenic ($P < 0.05$). When the calcine results were not included in the analysis and only slags and soils were statistically evaluated, these same two chemical extractants were still not significantly different from the *in-vivo* bioavailable arsenic ($P < 0.05$). In consideration of the calcine group of materials alone, they were significantly different from the *in-vivo* results. The ratio of arsenic extracted by sodium acetate or sodium hydroxide: *in-vivo* arsenic was much lower for calcine than for soil or slag materials. The chemical extractants which dissolve surficially complexed arsenic all underestimated the bioavailable arsenic fraction, while the two more aggressive arsenic extractants, the hydroxylamine hydrochloride and the ammonium oxalate, both greatly overestimated the bioavailable arsenic (Table 6). The sodium hydroxide extractant came closer to the true value of bioavailable arsenic, yet not close enough to be statistically significant.

To consider the variability of the various chemical extraction methods as compared to the variability of the *in-vivo* method, the coefficients of variation were determined for each (Table 6). In evaluating the chemical extractant results for all media, the CV values are all near or greater than 100%, except for the phosphate (40.8%) and ammonium oxalate (42.3%) extractants. The CV for the *in-vivo* method for all media is 65.6%. For the calcine material alone, the CVs for the chemical extractants range from 8.64% (ammonium oxalate) to 50.3% (hydroxylamine hydrochloride), while the *in-vivo* method demonstrates a CV of 88.9%. Evaluating all media except the calcine material, the CVs range from 21.7% (phosphate) to 155% (water), while the

in-vivo method CV is at its lowest at 49.1%. The generally large CVs show low precision with the chemical extractants which correspond to the relatively poor correlations (r^2) (Table 6).

From the data presented, it appears that the fraction of arsenic in contaminated soils and solid wastes which is bioavailable is comprised of arsenic fractions between the surficially complexed (desorbable) arsenic and arsenic associated with the iron/manganese/aluminum oxides. Because the stomach environment is acidic (pH 1.8) and anaerobic, it is likely that most forms of adsorbed and some of the iron and manganese oxide fractions of arsenic are dissolved. The amounts of extracted arsenic between surficially adsorbed and surficially adsorbed plus occluded in iron and manganese oxide fractions may be similar to the chemical forms of arsenic dissolved in the stomach. The extracted arsenic from our experiment followed the trend: ammonium oxalate, hydroxylamine hydrochloride > sodium hydroxide, *in-vivo* > sodium acetate > phosphate, water.

It is unlikely that one chemical extractant will measure the fraction of bioavailable arsenic in all contaminated soils under all conditions. However, a chemical extractant that is more closely related to bioavailable arsenic than total arsenic for most of the major groups of environmental media is desirable. Chemical extractants which more closely reflect the environment of the gastro-intestinal tract have been shown to be better estimators of bioavailable arsenic in contaminated soils and solid media (Rodriguez, 1998). The conditions or concentrations of the more aggressive chemical extractant methods, such as the hydroxylamine hydrochloride, may eventually be designed to provide closer estimates of the true bioavailable arsenic content of contaminated materials by better simulating the gastric environment.

Chemical extractant methods that correlate well with nutrient phytoavailability or crop yield are well established in soil science. However, chemical extractant methods can not extract plant

nutrients in the same physiochemical manner as plants. Strong correlation between nutrients measured by soil extractants and plant uptake of nutrients has allowed soil scientists to make reasonable predictions of plant available nutrients in soil and fertilizer recommendations (Amacher, 1996). Chemical extractants have the ability to extract most chemical pools of arsenic. Further research in this area will provide evaluation of important soil exposure pathways, including: incidental ingestion, inhalation, and dermal exposure. A more reasonable measurement of the bioavailable fraction of arsenic in contaminated soils and wastes will provide for more reasonable estimates of risks at hazardous waste sites and more cost effective remedial strategies.

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Table 1. Major element content and select properties of study media.

	Study Media														
Properties	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5
pH[1]	2.6	2.6	3.1	3.1	5.7	7.4	7.7	7.1	7.4	7.4	3.9	4.6	7.5	7.3	7.6
TOC (%)[2]	0.36	0.22	0.58	0.41	0.61	0.89	3.13	1.58	3.38	3.22	0.81	1.52	2.28	0.23	4.58
% < 2 um[3]	5.7	10.1	13.4	9.8	7.4	6.5	18.4	8.4	7.3	7.5	--	--	--	--	--
% < 50 um[3]	51.6	49.8	57.4	45.5	49.4	30.2	59.1	36.5	45.1	45.6	--	--	--	--	--
Soluble Anions[4]															
	mg kg^{-1}														
Chloride	2545	2950	1237	1079	1711	944	1172	2198	976	1129	4224	9868	912	2446	14171
Sulfate	158281	83119	23368	224253	219776	1308	3287	10509	2898	2529	221126	347176	2484	1360	9449
Nitrate	102.5	159.9	100.3	49.4	297.3	146.9	941.7	507.0	552.2	628.2	323.9	2471.3	53.8	77.5	875.9
Major Elements[5]															
	%														
Si	17.39	17.02	18.00	18.04	22.67	16.83	21.27	23.00	20.05	20.47	22.60	28.83	16.66	12.78	26.54
Al	1.19	1.17	1.60	1.80	3.02	2.43	3.62	3.20	2.64	2.74	7.56	4.64	1.73	2.48	3.56
Ca	1.20	0.68	0.41	2.90	2.86	12.15	9.64	8.57	7.50	6.07	2.43	3.98	12.07	13.99	8.43
Mg	0.16	0.16	0.16	0.22	0.46	1.13	1.53	1.19	1.00	0.95	0.80	0.65	1.33	0.83	1.78
Na	0.14	0.10	0.22	0.24	0.42	0.13	0.33	0.56	0.36	0.36	0.01	0.50	0.01	2.08	0.24
K	0.57	0.53	0.68	0.66	1.15	0.70	1.28	1.01	0.81	0.85	2.99	1.54	0.41	0.66	1.15
Fe	29.66	31.68	28.54	24.97	16.65	20.91	11.68	16.65	17.21	18.33	7.07	2.01	20.37	22.54	6.17
Mn	0.06	0.07	0.06	0.05	0.07	0.21	0.12	0.09	0.12	0.12	1.58	0.28	0.36	0.88	0.11
Ti	0.14	0.14	0.17	0.18	0.29	0.22	0.30	0.25	0.22	0.23	0.40	0.24	0.10	0.22	0.20
Heavy Metals[6]															
	mg kg^{-1}														
Pb	11072	12105	10983	8431	5528	8736	6835	3512	12612	11526	9196	214	11844	12061	3675
Cu	385	318	384	524	997	1807	1606	2208	4228	4009	975	8243	2212	2547	954
Ni	39.1	35.9	31.4	32.2	37.4	24.5	24.4	31.9	35.3	34.0	14.8	17.3	36.7	24.5	24.3

[1]1:1, soil:0.01MCaCl$_2$, [2]Total Organic Carbon (Nelson and Sommers, 1996), [3]Pipette Method (Gee and Bauder, 1986)

[4]1g soil:10 ml H_2O, Shake 1-hr, Filter 0.45 um, [5]X-Ray Fluor. (Karathanasis and Hajek, 1996), [6]SW 846, Method 3050 (USEPA, 1986)

Table 2. Chemical content of arsenic in study media.

Arsenic Concentration	Study Media														
	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5
Total (mg kg^{-1})[(1)]	11294	17456	13472	11525	6245	405	450	1180	5022	4650	331	233	799	1463	401
TCLP (mg L^{-1})[(2)]	3.0	3.0	3.0	2.9	3.0	3.0	2.8	2.9	3.2	2.9	2.6	2.8	2.6	2.7	2.9
TCLP (mg kg^{-1})[(2)]	59.4	60.3	60.5	58.8	60.0	59.2	55.6	58.1	63.4	58.6	53.0	55.1	52.2	54.5	59.0

[(1)]SW 846, Method 3050 (USEPA, 1986)

[(2)]SW 846 Method 1311 (USEPA, 1986)

Table 3. Arsenic chemical extractants and their respective literature references.

Chemical Extractant	Arsenic Species Extracted	Reference
Water	water soluble (WS)	Huang and Fujii, 1996
Sodium Acetate 1 M NaOAc, pH 5	WS + weakly adsorbed (WA)	Tessier et al., 1979
Phosphate 3 parts 0.1 M Na_2HPO_4 2 parts 0.1 M NaH_2PO_4	WS + WA + strongly adsorbed (SA)	Yamamoto, 1975
Hydroxylamine HCl 0.25 M NH_2OH, 0.25 M HCl, 0.025 M H_3PO_4	WS + WA + SA + amorphous Mn Oxide (MnO) + amorphous Fe Oxide (FeO)	Chao and Zhou, 1983; modified by Amacher and Kotuby-Amacher, 1994
Ammonium Oxalate 0.2 M $(NH_4)_2C_2O_4$ HCl, 0.25 M HCl, 0.1 M ascorbic acid	WS + WA + SA + crystalline and amorphous FeO + aluminum oxide (AlO)	Shuman, 1982
Sodium Hydroxide 0.1 N NaOH, 1M NaCl	WS + WA + SA + AlO	Olsen and Sommers, 1982

Table 4. Arsenic concentrations of study media by various extraction methods.

Soil	Water	Sodium Acetate	Phosphate	Hydroxylamine Hydrochloride	Ammonium Oxalate	Sodium Hydroxide	Total Method 3050
				mg kg^{-1}			
C1	1.13	41.6	117	1717	7454	5088	11294
C2	1.25	44.6	122	2930	12616	9093	17456
C3	0.60	43.7	151	3018	9047	7341	13472
C4	0.78	52.9	381	4930	8130	2641	11525
C5	0.14	35.0	224	3085	4951	1589	6245
S1	0.74	88.8	74.4	384	409	983	405
S2	2.54	58.1	81.6	413	546	1004	450
S3	1.76	81.1	88.1	701	1214	1181	1180
S4	4.68	127	172	2619	4030	1641	5022
S5	3.54	83.0	107	2940	3331	1503	4650
E1	0.08	32.6	70.0	186	461	1064	331
E2	1.66	40.4	79.9	207	454	1054	233
E3	0.16	56.2	121	725	591	976	800
E4	0.49	195	78.8	1214	438	2313	1463
E5	1.30	47.6	96.9	308	552	1373	401
Summary Statistics							
mean	1.39	68.6	131	1692	3615	2590	4995
median	1.13	52.9	107	1214	1214	1503	1463
minimum	0.08	32.6	70.0	186	409	976	233
maximum	4.68	195	381	4930	12616	9093	17456

Table 5. Comparison of chemically extracted arsenic with *in-vivo* bioavailable arsenic.

Soil	Water	Sodium Acetate	Phosphate	Hydroxylamine HCL	Ammonium Oxalate	Sodium Hydroxide	*In-Vivo*
	% bioavailable arsenic						
C1	0.010	0.36	1.04	15.2	66.0	9.01	2.7
C2	0.011	0.39	0.70	16.8	72.3	10.4	3.3
C3	0.005	0.38	1.12	22.4	67.2	10.9	8.3
C4	0.007	0.46	3.30	42.8	70.5	4.58	22.1
C5	0.001	0.30	3.59	49.4	79.3	5.09	30.1
S1	0.006	0.77	18.4	94.8	101	48.6	--
S2	0.051	1.16	18.1	91.9	121	44.6	--
S3	0.035	1.62	7.47	59.4	103	20.0	28.7
S4	0.093	2.54	3.42	52.1	80.2	6.53	30.1
S5	0.070	1.65	2.31	63.2	71.6	6.47	16.4
E1	0.023	13.6	21.2	56.2	139	64.3	6.2
E2	0.713	22.3	34.3	89.0	195	90.5	42.8
E3	0.021	7.92	15.1	90.7	73.9	24.4	29.1
E4	0.034	18.6	5.38	83.0	29.9	31.6	18.7
E5	0.324	15.4	24.2	76.9	138	68.5	36.5
Summary Statistics							
mean	0.09	5.83	10.64	60.2	93.9	29.7	21.2
median	0.02	1.62	5.38	59.4	79.3	20.0	22.1
minimum	0.00	0.30	0.70	15.2	29.9	4.58	2.70
maximum	0.71	22.3	34.3	94.8	195	90.5	42.8

Table 6. Analysis of variance using Duncan's Multiple Range Test (SAS, 1997). Values reported are % bioavailable arsenic (or extractable arsenic) means for that group. Means that are not different at $P < 0.05$ have the same letter.

Samples	Water	Sodium Acetate	Phosphate	Hydroxylamine HCl	Ammonium Oxalate	Sodium Hydroxide	*In-Vivo*
All Media (n=13)	0.15 d	7.16 cd	1.04 d	81.6 a	93.9 a	29.7 b	21.3 bc
CV*(%)	198	97.7	40.8	108	42.3	90.4	65.6
Calcine Only (n=5)	0.01 e	0.39 e	1.48 e	29.3 b	71.1 a	8.00 d	14.1 c
CV*(%)	46.2	28.1	29	50.3	8.64	34.7	88.8
All Media Except Calcine (n=8)	0.22 d	10.69 cd	0.82 d	107.7 a	105.3 a	40.6 b	25.9 bc
CV*(%)	155	57.8	21.7	90.4	42.2	66.4	49.1

*CV = Coefficient of variation, %

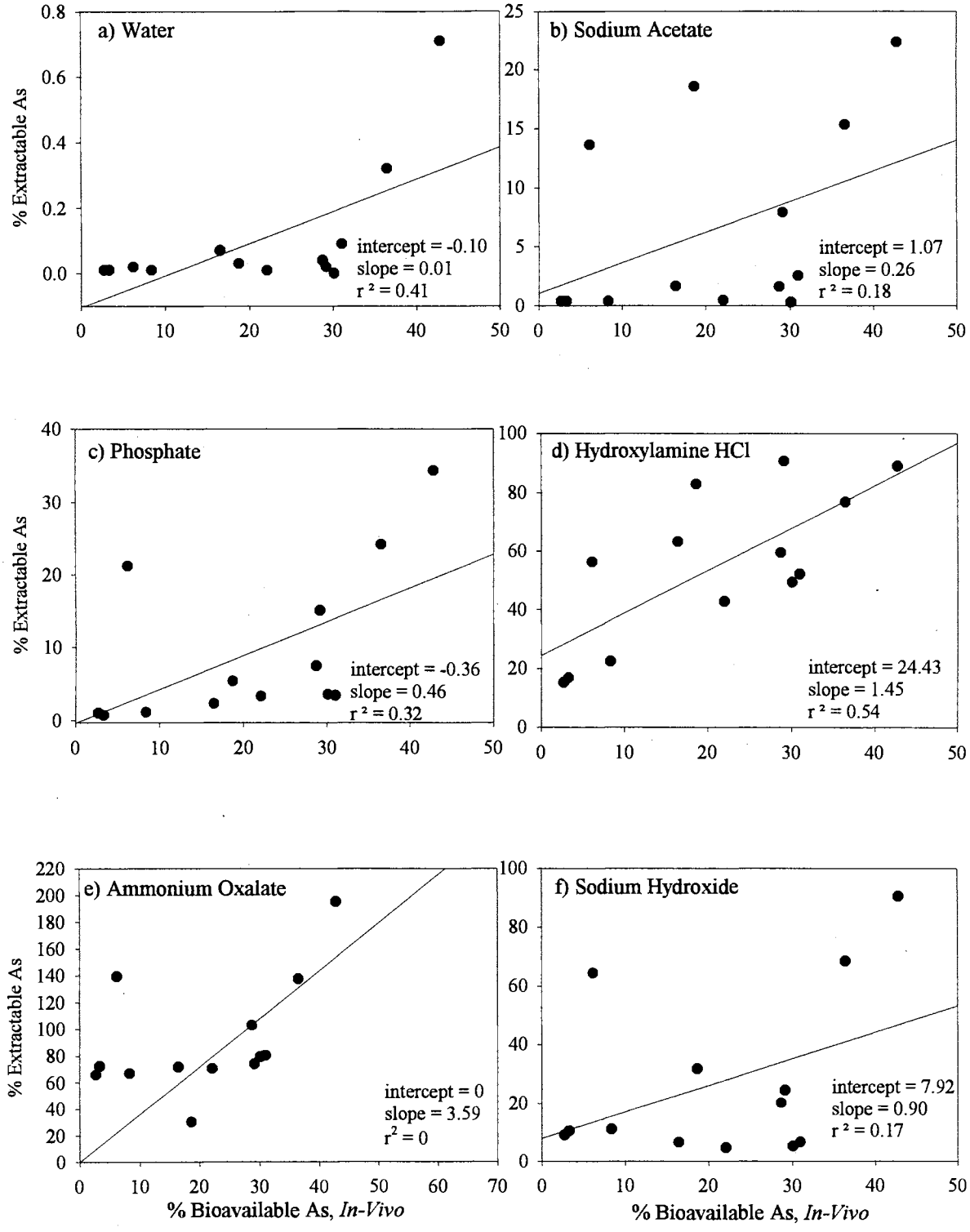

Figure 1. Linear regression correlations of *in-vivo* bioavailable As with As extracted by various chemical speciation methods.

Chapter 3

Biomethylation of Arsenic in Contaminated Soils and Solid Wastes

Abstract

Biomethylation of arsenic from soils is a naturally occurring phenomenon wherein indigenous fungi and bacteria methylate inorganic arsenic to form volatile, biomethylated forms of arsenic. Utilization of biomethylation as a remediation method for soils and wastes heavily contaminated with arsenic has not been shown. An experiment was conducted on calcine and lead slag waste materials (smelter wastes) high in total arsenic (10,920 and 3,160 mg kg^{-1}, respectively) and lead (8,430 and 12,610 mg kg^{-1}, respectively) concentrations to determine if biomethylation could be promoted as a remediation method to lower soil arsenic levels. Contaminated materials were supplemented with ground soybean meal (60,000 mg kg^{-1}), glutamine (1%), vermiculite (1:4, by volume), kept moist with distilled water and then incubated under aerobic and anaerobic conditions. After a 20 week incubation period, the calcine waste demonstrated 5.3 μg of arsenic lost via biomethylation (0.0005% of the total calcine arsenic). An experiment conducted on an uncontaminated soil spiked with a known amount of arsenic was performed to determine if the biomethylation conditions were adequate to promote biomethylation. Biomethylation resulted in 20.4 μg of arsenic volatilized over a 20 week period (0.002% of the total calcine arsenic). Another experiment was conducted to determine if the high lead concentrations inhibited the biomethylation process. The calcine waste material was supplemented with reagent grade lime to raise the pH to 7.4 and reduce the bioavailable fraction of lead. Biomethylation resulted in the formation of 96.8 μg of

volatilized arsenic (0.009% of the total calcine arsenic), a > 17-fold increase. Further optimization of this enhanced biomethylation technique may present a cost-effective remedial technology for some arsenic contaminated soils and solid wastes.

Key Words: bioremediation, biomethylation, arsenic contamination, bioavailable arsenic,

INTRODUCTION

As early as 1839, mysterious poisonings have been attributed to a volatile organic compound liberated from moldy wall-paper in damp rooms (Challenger *et al.*, 1933). Work by Gosio in 1839 led to the discovery that *Aspergillus sp.* and *Penicillium sp.* are responsible for the production of this gas (referred to as Gosio gas) via their production of hydrogen, which reduced the arsenic in the wall-paper pigments to hydrogen arsenide (Challenger *et al.* 1933). In 1945, Challenger published a comprehensive paper describing previous experiments which established that the primary component of the fatal Gosio gas was trimethylarsine formed from biomethylation of arsenic compounds by microorganisms. Challenger (1945) also showed that selenium and tellurite (also metalloids) were capable of being metabolized by fungi in a similar manner.

The volatile forms of arsenic produced via microbially mediated biomethylation include monomethyl arsine (MMA), dimethyl arsine (DMA), and trimethyl arsine (TMA). Several studies have shown that various microorganisms convert soil arsenic to the volatile forms: methanogenic bacteria, under anaerobic conditions (McBride and Wolfe, 1971; McBride *et al.*, 1978); the fungi *Candida humicola* (Cox and Alexander, 1974); wood-rotting fungi *Lenzites trabea* (Merrill and French, 1964); *Penicillium sp.*(Huysmans and

Frankenberger, 1991); *Scopulariopsis brevicaulis*, under aerobic conditions (McBride and Wolfe, 1971); and the marine algae *Tetraselmis chui* (Bottino *et al.*, 1978). Studies have been conducted to evaluate the production of arsine derivatives in soil (Cheng and Focht, 1979; Woolson 1977), sewage sludge (Cox and Alexander, 1973), surface waters (Braman and Foreback, 1973), and in humans (Braman and Foreback, 1973).

The mechanism for fungal biomethylation proposed by Cox and Alexander (1973) for the three species *Candida humicola, Gliocladium roseum,* and *Penicillium* sp. is as follows:

monomethylarsonic acid → dimethylarsinic acid → trimethylarsine oxide →TMA

In addition, *C. humicola* was also found to use arsenate and arsenite as substrates to produce trimethylarsine. According to Cullen *et al.* (1977), it is expected that another product of fungal biomethylation by these species would include DMA. However, the authors suggest that DMA would have a very short life time in an air stream, consequently, even if it were formed as a metabolic product under aerobic conditions it would be chemically transformed quickly to dimethylarsenic acid and then metabolized to TMA (Cullen *et al.*, 1977).

The biomethylation process requires a methyl-donor, or precursor (Cullen et al., 1977; Cullen and Reimer, 1989; Tamaki and Frankenberger, 1992). Methylation is thought to occur via S-adenosylmethionine (SAM) (Tamaki and Frankenberger, 1992). SAM, which is an "active" form of methionine, is thought to be involved in the transfer of the methionine methyl group to arsenic during fungal methylation (Cullen and Reimer, 1989).

Numerous species of bacteria have been found to methylate arsenic under both aerobic and anaerobic conditions. The most widely studied, *Methanobacterium* sp., has been shown to produce volatile DMA when incubated anaerobically with a variety of arsenic derivatives,

H_2, ATP, and a methyl donor (methylcobalamin) (McBride and Wolfe, 1971). TMA has not been shown to be an end product of methanogenic bacterial methylation.

Since 1971, a number of nonmethanogenic bacteria have been identified as methylarsine producers (Cullen and Reimer, 1989). The bacteria identified were first isolated from soils and sediments and acclimated to grow in arsenate at concentrations < 100 mg kg^{-1}. The bacterial cultures were exposed to sodium arsenate and sodium methylarsonate herbicides under aerobic conditions. The response noted was the production of DMA and TMA arsenic species.

Under laboratory conditions Hassler et al.. (1984) studied the biomethylation of organoarsenicals, dimethylarsinic acid and methanearsonic acid, when added to soil at the rate of 20 mg kg^{-1}. Crushed soybean meal, at the concentration of 60,000 mg kg^{-1}, was added to each soil as an organic matter source to provide carbon and nitrogen. Under aerobic conditions, the methylated arsenic products measured after a 12 week incubation period were: 3.37 mg kg^{-1} of dimethylarsinic acid, 0.75 mg kg^{-1} of methanearsonic acid, and 0.07 mg kg^{-1} of sodium arsenate. Anaerobic conditions were maintained in a separate experiment by continually flushing their reactors with nitrogen gas. Results of the anaerobic experiment at the end of 12 weeks were: 0.25 mg kg^{-1} dimethylarsinic acid and 0.009 mg kg^{-1} methanearsonic acid.

In another study (Akins and Lewis, 1976) soil was treated with 100 mg kg^{-1} of a labeled organoarsenical, disodium methanearsonate, and loss of soil arsenic was measured as a function of redox conditions, organic matter, and moisture content. The greatest loss (11.0%) was found under moist, anaerobic conditions with added organic matter. The moist,

aerobic conditions with added organic matter soil was found to lose 7.9%. Without added organic matter, 8.1% arsenic was lost under moist anaerobic conditions, while only 2.2% arsenic was lost under moist aerobic conditions.

Biomethylation of selenium, a metalloid similar to arsenic, has also been demonstrated experimentally. Abu-Erreish et al. (1968) measured methylated selenium (by fluorometric methods) produced by incubating soils containing 0.67 to 9.1 mg kg^{-1} natural selenium. Other researchers, using gas chromatography-mass spectrometry techniques have demonstrated that selenium in soil is biomethylated by indigenous microorganisms to form biomethylated end products, (eg. dimethyl selenide) when the soils are enhanced with organic materials under both aerobic and anaerobic conditions (Doran and Alexander, 1977; Reamer and Zoller, 1980; Cooke and Bruland, 1987;Karlson and Frankenberger, Jr., 1989; Thompson-Eagle and Frankenberger, Jr., 1990). A methyl-group supplying amino acid, L-methionine, was shown to enhance biomethylation of selenium at more than twice the rate of the control (Frankenberger and Karlson, 1989); an optimum concentration of L-methionine was determined to be 100 mg kg^{-1} in soil. Some metals have been demonstrated to inhibit biomethylation of selenium. Karlson and Frankenberger (1988) found that the addition of 5 mmol kg^{-1} of other metals, molybdenum, mercury, chromium, and lead, to seleniferous soils greatly inhibited selenium volatilization; whereas arsenic, boron, and manganese had little effect.

To date, there are no reports in the literature that describe the utilization of bioremediation as a technology to remediate arsenic contaminated soils. The research published to date has been demonstrated only on soils with low level arsenic contamination;

100 mg kg^{-1} and less. The primary objective of our study was to document biomethylation of inorganic arsenic would occur in materials heavily contaminated with arsenic and heavy metals. A second objective was to compare biomethylation under aerobic (using air) and anaerobic (using argon) conditions.

EXPERIMENTAL METHODS

Study Materials

Two matrices were collected for this study from a typical mining/smelter site in the western U.S. where wastes were deposited between 20 and 50 years ago. These aged and weathered wastes include a calcine material, a waste product which results from the roasting and smelting of arsenopyrite ore for the extraction of arsenic, and an iron slag material, a waste product which results from the smelting of ores for lead which is also high in iron. Approximately five gallons of each solid material was collected, air dried under ambient conditions, and sieved to collect the particle size fraction < 2 μm. Soils were thoroughly homogenized/mixed prior to use and stored in secured, air-tight containers. A background location was determined and five gallons of soil was collected and prepared in the same manner as the calcine and slag materials.

The chemical and physical properties of the calcine, slag, and background materials are presented in Table 1. Arsenic characterization of the two study materials is presented in Table 2. Total arsenic is extracted from the study materials by hot nitric acid/peroxide digestion (USEPA, 1986) and arsenic analysis was performed by a Thermo-Jarell Ash Inductively Coupled Plasma (ICP) (Maxim). However, the entire fraction of total arsenic will

not be available (or bioavailable) to the microorganisms responsible for biomethylation. To consider other pools of arsenic, a variety of chemical extractants were performed and are presented in Table 2.

Mineralogical composition of the calcine and slag were determined by microprobe analysis for the various iron and arsenic bearing compounds. The calcine was found to contain: 38% iron-manganese sulfate, 28% iron-arsenic-oxide, and 35% iron-manganese oxide. The slag was found to contain: 17% iron-manganese sulfate, 49% iron-arsenic oxide, 4% iron-manganese oxide, 30% lead-manganese oxide, and 2% slag.

Biomethylation Reactor Conditions

Arsenic contaminated soil/waste material, 100 g, was placed into a 500 ml Erlenmeyer vacuum flask, as depicted in Figure 1. Organic material (nutrient source) was added in the form of crushed soybean meal to achieve a concentration of 60,000 mg kg^{-1} (Woolson, 1976); 12 g to each flask. A methyl group-containing amino acid, 1.0 g glutamine was added as a source of methyl-precursor (Huysmans and Frankenberger, Jr., 1991). Vermiculite was added to each flask as a bulking agent at the ratio of 1 part vermiculite to 4 parts of soil. To create moist conditions, 25 ml distilled water was added to each flask.

Anaerobic or aerobic conditions were maintained during the biomethylation experiment. Aerobic conditions were maintained by continuously passing a stream of humidified breathing air (Hassler et al., 1984) into some of the bioreactors at a flow rate of approximately 1 L min^{-1} through Tygon and glass tubing held in place with neoprene stoppers (Figure 1). Anaerobic conditions were maintained in the other reactors in the same manner

using humidified argon gas, at the same flow rate. Volatile arsenic gas formed during incubation was flushed out of the flasks with the air or argon and into a polycarbonate test tube containing 50 ml of 0.1 M potassium iodide (KI) solution. A small amount of crystal iodine was added to the KI to maintain iodine in excess. Volatilized arsenic in the forms of monomethylarsine (MMA) and dimethylarsine (DMA) are trapped in KI/iodine solution (Woolson, 1977). A second tube of 50 ml KI/iodine was placed sequentially to trap any MMA or DMA not trapped in the first KI tube (Figure 1). In order to trap any trimethylarsine (TMA) formed during the biomethylation experiment, a preformed carbon adsorption tube (ORBO-100, SUPELCO, Bellefonte, PA) was positioned to adsorb TMA from the effluent air or argon.

The bioreactor flasks were housed in the dark in a temperature controlled room with the temperature maintained at 85°F throughout the experiment. The KI solutions and carbon adsorption tubes were collected and exchanged for new solutions and tubes on the following sampling schedule: 2, 4, 8, 12, 16, and 20 weeks.

Biomethylation Experimental Design

The biomethylation experiment was performed with the two arsenic contaminated study materials, lead slag and calcine, under both aerobic and anaerobic conditions. Four replicates of each treatment was performed. To demonstrate that biomethylation of soil arsenic occurs via microbially mediated mechanisms, sterilized controls (performed in duplicate) were included to evaluate abiotic arsenic losses. Sterilization was first accomplished by autoclaving. However, after two weeks of incubation, microbial mats were

visually observed in some of the control bioreactor flasks. A second sterilization was accomplished by adding a solution of $HgCl_2$ to achieve a concentration of 500 mg kg^{-1} in soil (Wolf and Skipper, 1994).

Analysis of Volatilized Arsenic

Arsenic trapped in the KI solutions was measured by utilizing the hydride generation (HG) technique with ICP. To prepare the KI solutions for hydride generation, a 10.0 ml aliquot of KI was placed into a test tube and mixed with 3.3 ml concentrated hydrochloric acid and 4.0 ml of a solution containing 10% KI and 1% ascorbic acid. After a reaction period of at least 1 hour, arsenic was determined by ICP-HG. During hydride generation, the sample solution is mixed with a solution of 1% $NaBH_4$ made up in 0.1 M NaOH, which results in the development of arsine gas. Arsenic was extracted from the carbon adsorbent by hot digestion with nitric acid, followed by hydrogen peroxide and hydrochloric acid as described in SW-846, Method 3050 (USEPA, 1986). The resulting extract was then analyzed by ICP-HG as described above for the KI solutions.

Positive Control Experiment

An experiment was conducted using the uncontaminated background soil to evaluate the biomethylation of a known amount (controlled spike) of arsenate. It was determined experimentally that adding 20 ml of a solution of sodium arsenate, 500 mg L^{-1} arsenic, shaking at low speed for 4 hours, filtering and allowing to air dry, resulted in a water soluble arsenic extract of 10 mg kg^{-1}. The positive control biomethylation experiment was performed

aerobically, in triplicate, and all reactor conditions as described for the biomethylation experiment were maintained. Samples of KI and adsorbing carbon were collected and analyzed on the same schedule as the above biomethylation experiment.

Lead Stabilized Calcine Experiment

To evaluate the effect of lead inhibition of the biomethylation process, a lead stabilizing experiment was conducted using the calcine waste material. The calcine material had a pH of 3.1 and total lead content of 8,430 mg kg^{-1} (Table 1). Lime, in the form of reagent grade $CaCO_3$, was added to the calcine at 10 g kg^{-1} to achieve a pH of 7.4. The biomethylation experiment, as described above, was conducted on the lead-stabilized calcine waste. Treatments were performed in triplicate under aerobic conditions. Samples of KI and the carbon adsorbents were collected and analyzed on the same schedule as the above biomethylation experiment.

RESULTS AND DISCUSSION

Biomethylation Experiment

The biomethylation experiment conducted on the lead slag and calcine waste materials was incubated for a period of 20 weeks. Cumulative volatile arsenic produced over this time period is presented in Figure 2. Results of MMA + DMA, TMA, and total arsenic are all presented separately in Figure 2. Only the calcine waste under aerobic conditions demonstrated appreciable amounts of methylated arsenic. Over the incubation period of 20 weeks, a total of 5.31 μg of arsenic was methylated (which represents 0.0005% of the total

calcine arsenic); 2.17 μg of MMA + DMA, 3.14 μg of TMA (Table 3). In the calcine sterilized control, 1.08 μg of total arsenic was methylated over the incubation period (which represents 0.0001% of the total calcine arsenic). As previously reported in the literature, under aerobic conditions, both fungi and bacteria biomethylate soil arsenic to MMA, DMA, and TMA (Cox and Alexander, 1973; Cullen et al., 1977). Our results for the aerobic calcine treatments are in agreement with these reports. However, the amounts of volatilized arsenic produced are quite low by comparison with previously published research. Hassler et al. (1984) produced 343 μg volatilized arsenic (5.1% of the total soil arsenic) from incubating soils aerobically (over a period of 12 weeks). Their original study soil had 47 mg kg^{-1} total arsenic, to which they added a dimethylarsinic acid at the rate of 20 mg kg^{-1} arsenic in soil.

Comparing the results of the calcine biomethylated arsenic under aerobic conditions with the arsenic concentrations presented in Table 2, we can evaluate which pool (or fraction) of arsenic is the bioavailable form. The mass of biomethylated arsenic produced, 5.31μg arsenic, corresponds to an arsenic concentration of 0.053 mg kg^{-1} (100 g of soil in the bioreactor). As shown in Table 2, the water soluble extracted arsenic content of the calcine waste material is represented by 0.73 mg kg^{-1}. Therefore, by subjecting the calcine waste to biomethylation conditions, we have only biomethylated a small portion of the water soluble extracted arsenic.

The anaerobic calcine biomethylation treatment was not substantially different from the sterilized control; there was very little biomethylation activity. The lead slag biomethylation results, under aerobic and anaerobic conditions, did not show appreciable amounts of methylated arsenic over the sterilized lead slag controls. Previous reports in the

literature have shown substantial amounts of volatilized arsenic produced under anaerobic conditions. Cheng and Focht (1979) measured 80 μg of volatilized arsenic (5% of the total soil arsenic) produced over a three week incubation period of soil amended with arsenate (160 mg kg^{-1}) and maintained under flooded conditions. Another experiment (Woolson, 1977) conducted under anaerobic conditions (reactors flushed with N_2 gas), with soils amended with arsenic to achieve a rate of 10 mg kg^{-1}, showed up to 78 μg of the amended arsenic (7.8% of the total soil arsenic) was converted to volatilized species after 160 days. Redox measurements were not reported for either of these two reports. Redox measurements in our study ranged from approximately -4 to -12 mV (pH 3) for the calcine bioreactors and from -6 to +25 mV (pH 7) for the lead slag bioreactors, which reflect anaerobic conditions but not methanogenic. Primary anaerobic microorganisms responsible for biomethylation in natural systems require a redox potential of < -250 mV to be methanogenic. Perhaps greater amounts of volatilized arsenic would have been produced in the anaerobic bioreactors if the redox potentials were low enough.

Because the experimental results of biomethylation of the lead slag and calcine waste materials do not compare well with the amounts of arsenic reported to be biomethylated under aerobic conditions in the literature, further evaluations were deemed necessary. The first objective was to determine if the biomethylation process as performed in the initial experiments could be demonstrated for an uncontaminated, clean background soil that had a known amount of soil arsenic. This was accomplished by adding a known amount of arsenate (spike) to a "clean" soil and incubating under the same experimental conditions. Second, because there are reports in the literature of the presence of lead inhibiting the

selenium biomethylation process (Karlson and Frankenberger, 1988), a biomethylation experiment on lead stabilized calcine was performed.

Positive Control Experiment

Sodium arsenate additions to background soil resulted in 10 mg kg^{-1} water soluble arsenic. Methylated arsenic produced over the 20 week incubation period was 20.4 μg (Figure 3a) (0.002% of the total calcine arsenic), which is a > 3-fold increase in the biomethylated arsenic produced from the untreated calcine waste (Table 3). The amount of biomethylated arsenic produced for the spiked background experiment (20.4 μg) is more comparable to the biomethylated arsenic reported in past reports of the scientific literature (Hassler et al ., 1984). However, given the high concentration of arsenic in the calcine material (8,430 mg kg^{-1}), it is likely that another condition present in the calcine may be inhibiting biomethylation.

Lead Stabilized Calcine Experiment

The chemical form of lead in smelter waste controls the bioavailability of lead (Gradwohl and Basta, 1998). Bioavailable lead is the form which can be readily assimilated and toxic to microorganisms. Treatment of the calcine media with alkaline amendments (i.e. $CaCO_3$) will raise the soil pH and convert bioavailable lead into insoluble metal precipitates, complexes, and secondary minerals (Pierzynski and Schwab, 1993). Treatment of the calcine media with $CaCO_3$ should reduce lead bioavailability and increase microbial activity and biomethylation of arsenic. Therefore, to determine if lead was inhibiting the biomethylation

process, the biomethylation experiment conditions were repeated on the lead stabilized calcine waste material (in triplicate).

The amount of total volatilized arsenic produced from biomethylation of the lead stabilized calcine waste material over a 20 week period was 96.8 μg arsenic (0.009% of the total calcine arsenic), nearly all from MMA + DMA (92.4 μg arsenic, or 95.4%) (Figure 3b). By stabilizing the lead in the original calcine waste material, an increase of > 17-fold of biomethylated arsenic was produced.

The amount of biomethylated arsenic from the lead stabilized calcine waste (96.8μg) equates to a soil arsenic content of 0.97 mg kg^{-1}. Comparing this amount of biomethylated arsenic with the various pools of arsenic extracted from the calcine (as shown on Table 2) suggests the pool of arsenic available for biomethylation under these conditions falls between the water soluble plus weakly adsorbed arsenic (WA+WS) and the pool of arsenic more strongly adsorbed to the soil particles (WA+WS+SA).

It is important to note that all the biomethylation experiments were conducted over a 20 week period and were not re-supplied with nutrients or methyl-precursor at any other time. The low amounts of arsenic biomethylated in later time periods may be indicative of a deficiency of nutrients or methyl groups, rather than exhausting the bioavailable pool of arsenic. While the biomethylation results are low in concentration as compared to the total arsenic content, this bioremediation technique may be well-suited for areas which are difficult to treat by other, more traditional remedial technologies. With the prevalence of indigenous microorganisms responsible for methylating soil arsenic, it is certain that these processes are occurring naturally in areas where the appropriate environmental conditions exist, perhaps as

an example of "intrinsic bioremediation". Further research may be designed to optimize biomethylation of arsenic in contaminated soils and wastes to result in a cost-effective, natural remediation technology.

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Table 1. Elemental content and select chemical properties of study media. 80

Properties	Study Media		
	Calcine	Slag	Background Soil
pH[1]	3.1	7.1	7.3
TOC (%)[2]	0.51	2.87	0.54
% < 2 um[3]	9.8	7.3	12.2
% < 50 um[3]	45.5	45.1	55.2
Soluble Anions[4]			
	---------- mg kg^{-1} ----------		
Chloride	3029	10530	855
Sulfate	947	225383	1978
Nitrate	753	291	291
Major Elements[5]			
	---------- % ----------		
Si	18.1	19.9	28.0
Al	1.38	2.34	5.09
Ca	3.34	9.22	6.26
Mg	0.19	0.97	1.60
Na	0.19	0.30	0.82
K	0.56	0.66	1.91
Fe	26.6	19.7	4.56
Mn	0.05	0.12	0.05
Ti	0.14	0.20	0.41
Heavy Metal Contaminants[6]			
	---------- mg kg^{-1} ----------		
Pb	8430	12610	--
Zn	1660	4040	--
Cu	524	4230	--
Ni	32.2	35.3	--

[1]1:1, soil:0.01 M $CaCl_2$

[2]Total Organic Carbon (Nelson and Sommers, 1996)

[3]Hydrometer Method (Gee and Bauder, 1986)

[4]1 g soil:10 ml H_2O, Shake 1 hr.

[5]X-Ray Fluorescence (Karathanasis and Hajek, 1996)

[6]SW 846, Method 3050 (USEPA, 1986)

Table 2. Arsenic characterization of study media.

Method	Reference	Arsenic Fraction Extracted	Study Media		
			Calcine	Slag	Background
			mg kg^{-1}		
Water	Huang and Fujii, 1996	water soluble (WS)	0.73	3.81	0.31
Sodium Acetate 1M NaOAc, pH 5	Tessier et al., 1979	WS + weakly adsorbed (WA).	41.3	108	32.9
Phosphate 3 parts 0.1 M Na_2HPO_4 2 parts 0.1 M NaH_2PO_4	Yamamoto, 1975	WS + WA + strongly adsorbed (SA)	255	98.5	61.7
Hydroxylamine HCl 0.25 M NH_2OH, 0.25 M HCl, 0.025 M H_3PO_4	Chao and Zhou, 1983; modified by Amacher and Kotuby-Amacher, 1994	WS + WA + SA + amorphous Mn oxide (MnO) + amorphous Fe oxide (FeO)	375	213	33.2
Ammonium Oxalate 0.2 M $(NH_4)_2C_2O_4$ HCl, 0.25 M HCl, 0.025 M H_3PO_4	Shuman, 1982	WS + WA + SA + crystalline and amorphous FeO + aluminum oxide (AlO)	5403	2135	288
Sodium Hydroxide 0.1 N NaOH, 1M NaCl	Olsen and Sommers, 1982	WS + WA + SA + AlO	188	191	--
Hot Acid Digestion	SW 846, Method 3050 (USEPA, 1986)	all of the above + residual	10920	3160	69.2

Table 3. Summarized results of arsenic spiked background soil and lead stabilized calcine biomethylation as compared to calcine biomethylation. Aerobic conditions, 20 weeks of incubation. Mean values are presented.

Study Soil	Volatilized Arsenic		
	MMA+DMA	TMA	Total
	ug		
Calcine	2.2	3.1	5.3
Positive Control, Spiked Background Soil	19.8	0.58	20.4
Lead Stabilized Calcine	92.4	4.4	96.8

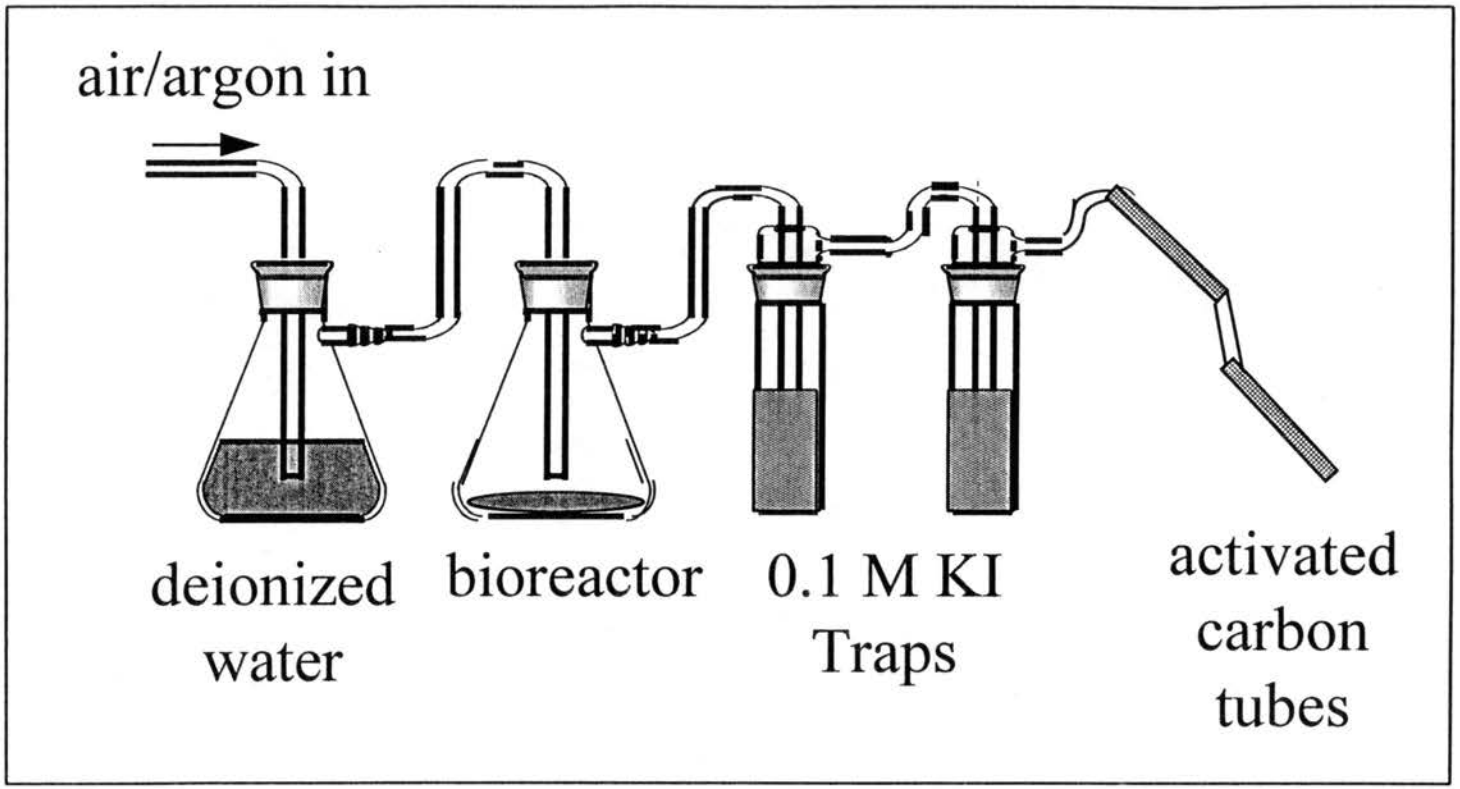

Figure 1. Bioreactor design.

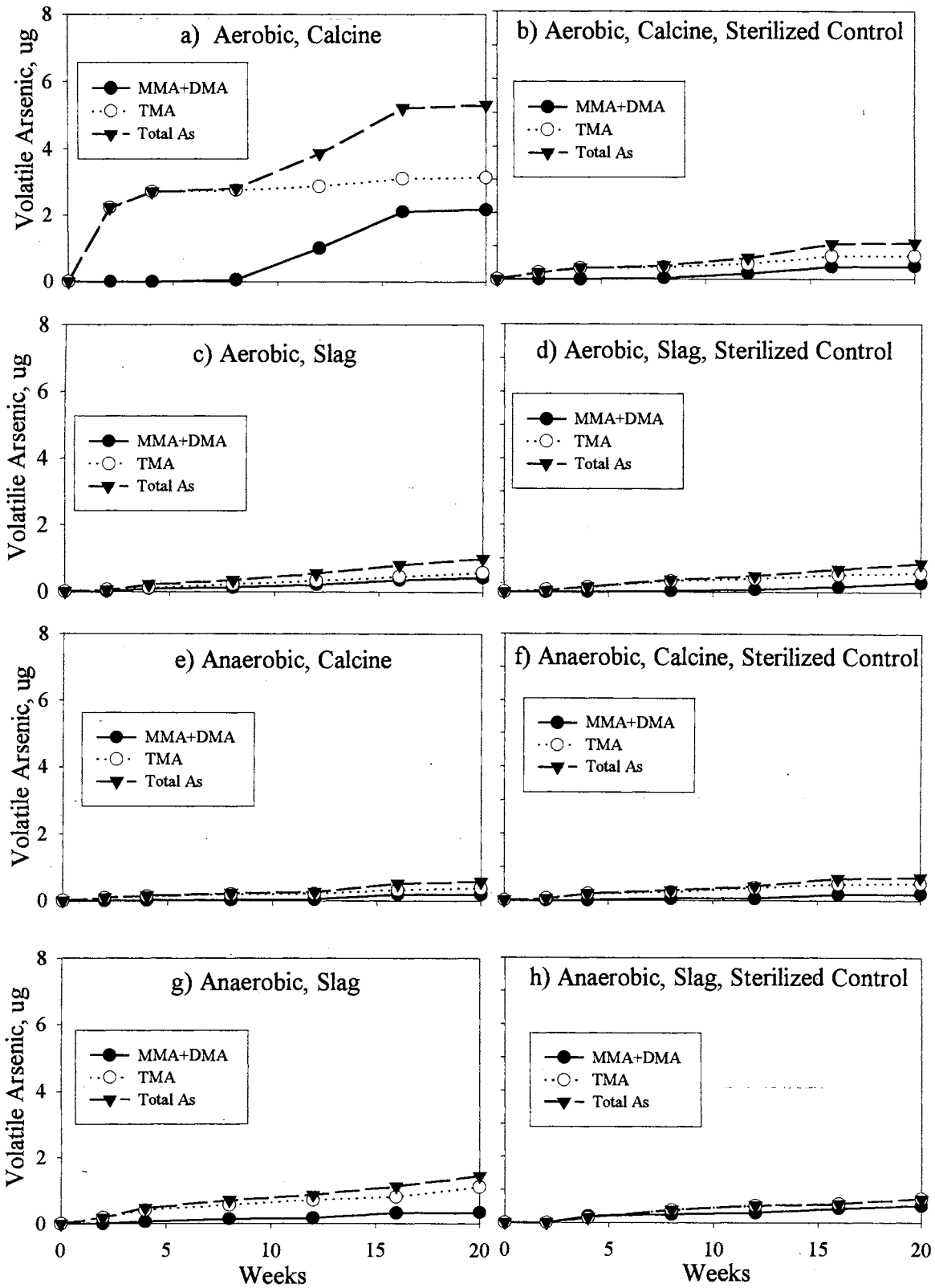

Figure 2. Volatile arsenic biomethylated from contaminated media under aerobic or anaerobic conditions.

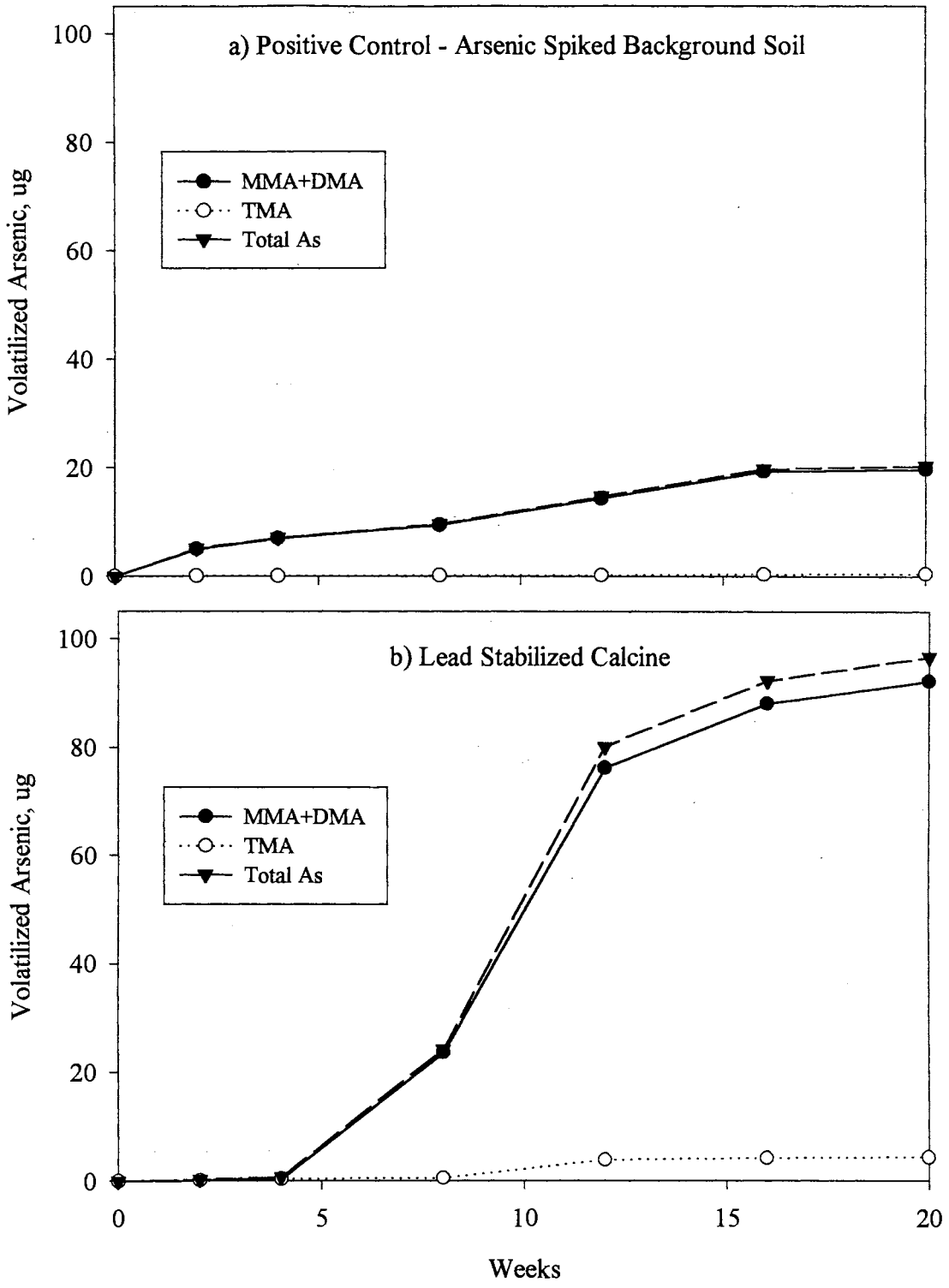

Figure 3. Volatilized (methylated) arsenic produced over a 20 week incubation period; positive control and lead stabilized calcine experiments.

Appendix I

In-Vitro Data

Table I - 1
Ruby In-Vitro (PBET) Method - Time Study

Sample No.	Sample ID	GI Phase Time (min)	Rep No.	As (ug/L)	Solution Dil'n Factor	As (ug/L)	As (mg/L)	As (mg/kg)	Ave As (mg/kg)	Bioavailable As (%)	std dev	Average
1429	C4	S-20	1	655.496	2	1310.992	1.311	131.099	126.75	1.14	0.04	1.10
1430	C4	S-20	2	613.658	2	1227.316	1.227	122.732		1.06		
1431	C4	S-20	3	632.025	2	1264.05	1.264	126.405		1.10		
1432	C4	S-40	1	982.300	2	1964.6	1.965	196.460	192.77	1.70	0.08	1.67
1433	C4	S-40	2	913.940	2	1827.88	1.828	182.788		1.59		
1434	C4	S-40	3	995.318	2	1990.636	1.991	199.064		1.73		
1435	C4	S-60	1	1093.324	2	2186.648	2.187	218.665	213.94	1.90	0.11	1.86
1436	C4	S-60	2	1000.127	2	2000.254	2.000	200.025		1.74		
1437	C4	S-60	3	1115.646	2	2231.292	2.231	223.129		1.94		
1438	C4	I-60	1	1171.615	2	2343.23	2.343	234.323	232.00	2.03	0.02	2.01
1438	C4	I-60	2	1150.356	2	2300.712	2.301	230.071		2.00		
1440	C4	I-60	3	1157.976	2	2315.952	2.316	231.595		2.01		
1441	C4	I-120	1	1225.761	2	2451.522	2.452	245.152	247.90	2.13	0.07	2.15
1442	C4	I-120	2	1210.206	2	2420.412	2.420	242.041		2.10		
1443	C4	I-120	3	1282.595	2	2565.19	2.565	256.519		2.23		
1444	C4	I-180	1	1257.266	2	2514.532	2.515	251.453	262.05	2.18	0.08	2.27
1445	C4	I-180	2	673.885	4	2695.54	2.696	269.554		2.34		
1446	C4	I-180	3	662.824	4	2651.296	2.651	265.130		2.30		
1447	S4	S-20	1	222.379	40	8895.16	8.895	889.516	784.29	17.71	1.86	15.62
1448	S4	S-20	2	188.120	40	7524.8	7.525	752.480		14.98		
1449	S4	S-20	3	177.717	40	7108.68	7.109	710.868		14.16		
1450	S4	S-40	1	217.743	40	8709.72	8.710	870.972	694.22	17.34	4.16	13.82
1451	S4	S-40	2	115.869	40	4634.76	4.635	463.476		9.23		
1452	S4	S-40	3	187.055	40	7482.2	7.482	748.220		14.90		
1453	S4	S-60	1	216.116	40	8644.64	8.645	864.464	704.93	17.21	3.95	14.04
1454	S4	S-60	2	120.643	40	4825.72	4.826	482.572		9.61		
1455	S4	S-60	3	191.940	40	7677.6	7.678	767.760		15.29		

Table I - 1
Ruby In-Vitro (PBET) Method - Time Study

Sample No.	Sample ID	GI Phase Time (min)	Rep No.	As (ug/L)	Solution Dil'n Factor	As (ug/L)	As (mg/L)	As (mg/kg)	Ave As (mg/kg)	Bioavailable As (%)	std dev	Average
1456	S4	I-60	1	141.855	40	5674.2	5.674	567.420	511.81	11.30	1.82	10.19
1457	S4	I-60	2	101.522	40	4060.88	4.061	406.088		8.09		
1458	S4	I-60	3	140.479	40	5619.16	5.619	561.916		11.19		
1459	S4	I-120	1	150.726	40	6029.04	6.029	602.904	531.67	12.01	1.89	10.59
1460	S4	I-120	2	105.930	40	4237.2	4.237	423.720		8.44		
1461	S4	I-120	3	142.093	40	5683.72	5.684	568.372		11.32		
1462	S4	I-180	1	142.750	40	5710	5.710	571.000	508.37	11.37	1.74	10.12
1463	S4	I-180	2	102.076	40	4083.04	4.083	408.304		8.13		
1464	S4	I-180	3	136.452	40	5458.08	5.458	545.808		10.87		
1465	S1	S-20	1	25.949	40	1037.96	1.038	103.796	109.39	25.88	1.66	27.01
1466	S1	S-20	2	29.192	40	1167.68	1.168	116.768		29.12		
1467	S1	S-20	3	26.904	40	1076.16	1.076	107.616		26.84		
1468	S1	S-40	1	29.013	40	1160.52	1.161	116.052	120.82	28.94	1.59	29.83
1469	S1	S-40	2	32.015	40	1280.6	1.281	128.060		31.94		
1470	S1	S-40	3	29.590	40	1183.6	1.184	118.360		29.52		
1471	S1	S-60	1	30.777	40	1231.08	1.231	123.108	134.37	30.70	2.69	33.18
1472	S1	S-60	2	36.141	40	1445.64	1.446	144.564		36.05		
1743	S1	S-60	3	33.860	40	1354.4	1.354	135.440		33.78		
1474	S1	I-60	2	5.658	40	226.32	0.226	22.632	23.23	5.64	1.06	5.74
1475	S1	I-60	3	5.957	40	238.28	0.238	23.828		5.94		
1476	S1	I-120	2	3.985	40	159.4	0.159	15.940	15.97	3.98	0.24	3.94
1477	S1	I-120	3	3.999	40	159.96	0.160	15.996		3.99		
1478	S1	I-180	2	3.583	40	143.32	0.143	14.332	14.65	3.57	0.11	3.62
1479	S1	I-180	3	3.740	40	149.6	0.150	14.960		3.73		

Table I - 2
Ruby In-Vitro Method (PBET)

Sample No.	Sample ID	GI Phase	Rep No.	As (mg/L)	Corrected As (mg/L)	Std Dev	As (mg/kg)	Ave As (mg/kg)	Bioavailable A %	Bioavailable A average	3050 As mg/kg
2309	C1	S	1	0.866	0.884	0.084	88.363	76.37	0.78	0.68	11294
2310	C1	S	2	0.632	0.644		64.413		0.57		11294
2311	C1	S	3	0.749	0.763		76.347		0.68		11294
2312	C1	I	1	1.000	1.020	0.092	102.000	100.48	0.90	0.89	11294
2313	C1	I	2	0.888	0.906		90.596		0.80		11294
2314	C1	I	3	1.067	1.088		108.834		0.96		11294
2315	C2	S	1	1.184	1.208	0.274	120.768	89.22	0.69	0.51	17456
2316	C2	S	2	0.737	0.751		75.123		0.43		17456
2317	C2	S	3	0.704	0.718		71.777		0.41		17456
2318	C2	I	1	1.269	1.294	0.589	129.438	143.47	0.74	0.82	17456
2319	C2	I	2	2.040	2.081		208.080		1.19		17456
2320	C2	I	3	0.911	0.929		92.881		0.53		17456
2321	C3	S	1	1.619	1.651	0.365	165.138	126.44	1.23	0.94	13472
2322	C3	S	2	1.193	1.217		121.686		0.90		13472
2323	C3	S	3	0.907	0.925		92.504		0.69		13472
2324	C3	I	1	1.783	1.819	0.149	181.866	165.48	1.35	1.23	13472
2325	C3	I	2	1.587	1.619		161.874		1.20		13472
2326	C3	I	3	1.497	1.527		152.694		1.13		13472
2327	C5	S	1	2.051	2.092	0.629	209.202	201.04	3.35	3.22	6245
2328	C5	S	2	1.318	1.344		134.436		2.15		6245
2329	C5	S	3	2.544	2.595		259.488		4.16		6245

Table I - 2
Ruby In-Vitro Method (PBET)

Sample No.	Sample ID	GI Phase	Rep No.	As (mg/L)	Corrected As (mg/L)	Std Dev	As (mg/kg)	Ave As (mg/kg)	Bioavailable A %	Bioavailable A average	3050 As mg/kg
2330	C5	I	1	1.835	1.872	0.319	187.170	150.72	3.00	2.41	6245
2331	C5	I	2	1.343	1.370		136.986		2.19		6245
2332	C5	I	3	1.255	1.280		128.010		2.05		6245
2333	S1	S	1	1.691	1.725	0.156	172.482	159.73	42.59	39.44	405
2334	S1	S	2	1.395	1.423		142.290		35.13		405
2335	S1	S	3	1.612	1.644		164.424		40.60		405
2336	S1	I	1	0.658	0.672	0.290	67.157	60.16	16.58	14.85	405
2337	S1	I	2	0.834	0.850		85.048		21.00		405
2338	S1	I	3	0.277	0.283		28.264		6.98		405
2339	S2	S	1	1.878	1.916	0.366	191.556	197.57	42.57	43.91	450
2340	S2	S	2	1.611	1.643		164.322		36.52		450
2341	S2	S	3	2.322	2.368		236.844		52.63		450
2342	S2	I	1	0.662	0.675	0.156	67.534	76.55	15.01	17.01	450
2343	S2	I	2	0.927	0.675		67.534		15.01		450
2344	S2	I	3	0.466	0.946		94.574		21.02		450
2345	S3	S	1	2.777	0.475	1.237	47.522	187.10	4.03	15.86	1180
2346	S3	S	2	2.260	2.833		283.254		24.00		1180
2347	S3	S	3	2.39	2.305		230.520		19.54		1180
2348	S3	I	1	1.940	1.979	0.026	197.880	195.02	16.77	16.53	1180
2349	S3	I	2	1.906	1.944		194.412		16.48		1180
2350	S3	I	3	1.890	1.928		192.780		16.34		1180

Table I - 2
Ruby In-Vitro Method (PBET)

Sample No.	Sample ID	GI Phase	Rep No.	As (mg/L)	Corrected As (mg/L)	Std Dev	As (mg/kg)	Ave As (mg/kg)	Bioavailable A %	Bioavailable A average	3050 As mg/kg
2351	S5	S	1	5.600	5.712	0.467	571.200	544.00	12.28	11.70	4650
2352	S5	S	2	4.805	4.901		490.110		10.54		4650
2353	S5	S	3	5.595	5.707		570.690		12.27		4650
2354	S5	I	1	4.350	4.437	0.117	443.700	430.24	9.54	9.25	4650
2355	S5	I	2	4.146	4.229		422.892		9.09		4650
2356	S5	I	3	4.158	4.241		424.116		9.12		4650
2357	E1	S	1	0.135	0.138	0.028	13.790	16.88	4.17	5.10	331
2358	E1	S	2	0.172	0.176		17.575		5.31		331
2359	E1	S	3	0.189	0.193		19.278		5.82		331
2360	E1	I	1	0.180	0.183	0.130	18.309	25.04	5.53	7.56	331
2361	E1	I	2	0.393	0.401		40.066		12.10		331
2362	E1	I	3	0.164	0.167		16.738		5.06		331
2363	E2	S	1	0.496	0.506	0.069	50.592	46.82	21.71	20.09	233
2364	E2	S	2	0.381	0.388		38.821		16.66		233
2365	E2	S	3	0.500	0.510		51.041		21.91		233
2366	E2	I	1	0.336	0.342	0.163	34.241	46.35	14.70	19.89	233
2367	E2	I	2	0.636	0.648		64.821		27.82		233
2368	E2	I	3	0.392	0.400		39.984		17.16		233
2369	E3	S	1	1.927	1.966	0.184	196.554	214.27	24.57	26.78	800
2370	E3	S	2	2.088	2.130		212.976		26.62		800
2371	E3	S	3	2.287	2.333		233.274		29.16		800

Table I - 2
Ruby In-Vitro Method (PBET)

Sample No.	Sample ID	GI Phase	Rep No.	As (mg/L)	Corrected As (mg/L)	Std Dev	As (mg/kg)	Ave As (mg/kg)	Bioavailable A %	Bioavailable A average	3050 As mg/kg
2372	E3	I	1	0.8358	0.853	0.114	85.252	80.59	10.66	10.07	800
2373	E3	I	2	0.6626	0.676		67.585		8.45		800
2374	E3	I	3	0.872	0.889		88.944		11.12		800
2375	E4	S	1	2.375	2.423	0.453	242.250	221.71	16.56	47.89	1463
2376	E4	S	2	1.665	1.698		169.830		11.61		1463
2377	E4	S	3	2.481	2.531		253.062		17.30		1463
2378	E4	I	1	1.288	1.314	0.087	131.376	125.49	8.98	8.58	1463
2379	E4	I	2	1.132	1.155		115.464		7.89		1463
2380	E4	I	3	1.271	1.296		129.642		8.86		1463
2381	E5	S	1	1.365	1.392	0.265	139.230	150.11	34.72	37.43	401
2382	E5	S	2	1.282	1.308		130.764		32.61		401
2383	E5	S	3	1.768	1.803		180.336		44.97		401
2384	E5	I	1	0.7628	0.778	0.126	77.806	71.97	19.40	17.95	401
2385	E5	I	2	0.7905	0.806		80.631		20.11		401
2386	E5	I	3	0.5634	0.575		57.467		14.33		401
2387	Ruby Blank			0.1048	0.107						

Table I - 3
In-Vitro Gastrointestinal Method (IVG)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	Bioavailable As std dev	Bioavailable As average	3050 As mg/kg
2388	C1	S	1	1.292	1.318	0.791	0.714	197.676	178.60	18.08	1.75	0.16	1.58	11294
2389	C1	S	2	1.057	1.078	0.647		161.721			1.43			
2390	C1	S	3	1.153	1.176	0.706		176.409			1.56			
2391	C1	I	1	0.928	0.947	0.568	0.562	142.015	140.39	2.76	1.26	0.02	1.24	
2392	C1	I	2	0.928	0.946	0.568		141.953			1.26			
2393	C1	I	3	0.897	0.915	0.549		137.210			1.21			
2394	C2	S	1	1.093	1.115	0.669	0.682	167.229	170.44	2.79	0.96	0.02	0.98	17456
2395	C2	S	2	1.126	1.149	0.689		172.278			0.99			
2396	C2	S	3	1.123	1.145	0.687		171.819			0.98			
2397	C2	I	1	1.122	1.144	0.687	0.683	171.666	170.70	6.40	0.98	0.04	0.98	
2398	C2	I	2	1.071	1.092	0.655		163.863			0.94			
2399	C2	I	3	1.154	1.177	0.706		176.562			1.01			
2400	C3	S	1	2.019	2.059	1.236	1.189	308.907	297.33	11.40	2.29	0.08	2.21	13472
2401	C3	S	2	1.870	1.907	1.144		286.110			2.12			
2402	C3	S	3	1.941	1.980	1.188		296.973			2.20			
2403	C3	I	1	1.906	1.944	1.166	1.199	291.618	299.83	15.16	2.16	0.11	2.23	
2404	C3	I	2	2.074	2.115	1.269		317.322			2.36			
2405	C3	I	3	1.899	1.937	1.162		290.547			2.16			
2406	C4	S	1	4.484	4.574	2.744	2.752	686.052	687.94	4.66	5.95	0.04	5.97	11525
2407	C4	S	2	4.531	4.622	2.773		693.243			6.02			
2408	C4	S	3	4.474	4.563	2.738		684.522			5.94			
2409	C4	I	1	4.438	4.527	2.716	2.659	679.014	664.79	19.82	5.89	0.17	5.77	
2410	C4	I	2	4.400	4.488	2.693		673.200			5.84			
2411	C4	I	3	4.197	4.281	2.569		642.141			5.57			
2412	C5	S	1	3.117	3.179	1.908	1.888	476.901	471.95	4.82	7.64	0.08	7.56	6245
2413	C5	S	2	3.054	3.115	1.869		467.262			7.48			
2414	C5	S	3	3.083	3.145	1.887		471.699			7.55			

Table I - 3
In-Vitro Gastrointestinal Method (IVG)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	Bioavailable As std dev	Bioavailable As average	3050 As mg/kg
2415	C5	I	1	3.059	3.120	1.872	1.841	468.027	460.28	8.98	7.49	0.14	7.37	
2416	C5	I	2	3.022	3.082	1.849		462.366			7.40			
2417	C5	I	3	2.944	3.003	1.802		450.432			7.21			
2418	S1	S	1	0.728	0.742	0.445	0.434	111.369	108.52	6.91	27.50	1.71	26.79	405
2419	S1	S	2	0.658	0.671	0.403		100.643			24.85			
2420	S1	S	3	0.742	0.757	0.454		113.541			28.03			
2421	S1	I	1	0.647	0.660	0.396	0.364	99.022	91.04	6.92	24.45	1.71	22.48	
2422	S1	I	2	0.570	0.581	0.349		87.149			21.52			
2423	S1	I	3	0.568	0.580	0.348		86.935			21.47			
2424	S2	S	1	1.106	1.128	0.677	0.695	169.218	173.76	16.46	37.60	3.66	38.61	450
2425	S2	S	2	1.046	1.067	0.640		160.038			35.56			
2426	S2	S	3	1.255	1.280	0.768		192.015			42.67			
2427	S2	I	1	0.843	0.860	0.516	0.607	129.010	151.63	19.61	28.67	4.36	33.70	
2428	S2	I	2	1.059	1.080	0.648		162.027			36.01			
2429	S2	I	3	1.071	1.092	0.655		163.863			36.41			
2430	S3	S	1	2.611	2.663	1.598	1.469	399.483	367.15	32.67	33.85	2.77	31.11	1180
2431	S3	S	2	2.184	2.228	1.337		334.152			28.32			
2432	S3	S	3	2.404	2.452	1.471		367.812			31.17			
2433	S3	I	1	2.550	2.601	1.561	1.349	390.150	337.37	45.91	33.06	3.89	28.59	
2434	S3	I	2	2.005	2.045	1.227		306.765			26.00			
2435	S3	I	3	2.060	2.101	1.261		315.180			26.71			
2436	S4	S	1	10.250	10.455	6.273	5.146	1568.250	1286.58	243.96	31.23	4.86	25.62	5022
2437	S4	S	2	7.465	7.614	4.569		1142.145			22.74			
2438	S4	S	3	7.512	7.662	4.597		1149.336			22.89			
2439	S4	I	1	9.628	9.821	5.892	4.789	1473.084	1197.17	239.15	29.33	4.76	23.84	
2440	S4	I	2	6.987	7.127	4.276		1069.011			21.29			
2441	S4	I	3	6.859	6.996	4.198		1049.427			20.90			

Table I - 3
In-Vitro Gastrointestinal Method (IVG)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	Bioavailable As std dev	Bioavailable As average	3050 As mg/kg
2442	S5	S	1	6.705	6.839	4.103	3.312	1025.865	827.99	171.38	22.06	3.69	17.81	4650
2443	S5	S	2	4.753	4.848	2.909		727.209			15.64			
2444	S5	S	3	4.777	4.873	2.924		730.881			15.72			
2445	S5	I	1	6.390	6.518	3.911	2.901	977.670	725.32	227.24	21.03	4.89	15.60	
2446	S5	I	2	3.509	3.579	2.148		536.877			11.55			
2447	S5	I	3	4.323	4.409	2.646		661.419			14.22			
2448	E1	S	1	0.232	0.236	0.142	0.126	35.450	31.60	3.82	10.71	1.15	9.55	331
2449	E1	S	2	0.182	0.185	0.111		27.815			8.40			
2450	E1	S	3	0.206	0.210	0.126		31.533			9.53			
2451	E1	I	1	0.203	0.207	0.124	0.113	31.044	28.35	2.38	9.38	0.72	8.57	
2452	E1	I	2	0.173	0.177	0.106		26.530			8.02			
2453	E1	I	3	0.180	0.183	0.110		27.479			8.30			
2454	E2	S	1	0.636	0.649	0.389	0.329	97.277	82.15	13.57	41.75	5.82	35.26	233
2455	E2	S	2	0.464	0.474	0.284		71.053			30.49			
2456	E2	S	3	0.511	0.521	0.312		78.107			33.52			
2457	E2	I	1	0.473	0.482	0.289	0.255	72.354	63.79	7.45	31.05	3.20	27.38	
2458	E2	I	2	0.393	0.401	0.241		60.190			25.83			
2459	E2	I	3	0.385	0.392	0.235		58.829			25.25			
2460	E3	S	1	1.693	1.727	1.036	1.040	259.029	260.05	1.00	32.38	0.12	32.51	800
2461	E3	S	2	1.7	1.734	1.040		260.100			32.51			
2462	E3	S	3	1.706	1.740	1.044		261.018			32.63			
2463	E3	I	1	1.453	1.482	0.889	0.906	222.309	226.39	6.29	27.79	0.79	28.30	
2464	E3	I	2	1.459	1.488	0.893		223.227			27.90			
2465	E3	I	3	1.527	1.558	0.935		233.631			29.20			
2466	E4	S	1	0.839	0.856	0.513	0.499	128.367	124.63	3.38	8.77	0.23	8.52	1463
2467	E4	S	2	0.7961	0.812	0.487		121.803			8.33			
2468	E4	S	3	0.8086	0.825	0.495		123.716			8.46			

Table I - 3
In-Vitro Gastrointestinal Method (IVG)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	Bioavailable As std dev	Bioavailable As average	3050 As mg/kg
2469	E4	I	1	0.6786	0.692	0.415	0.430	103.826	107.38	3.94	7.10	0.27	7.34	
2470	E4	I	2	0.6974	0.711	0.427		106.702			7.29			
2471	E4	I	3	0.7295	0.744	0.446		111.614			7.63			
2472	E5	S	1	1.099	1.121	0.673	0.615	168.147	153.77	12.89	41.93	3.21	38.35	401
2473	E5	S	2	0.9797	0.999	0.600		149.894			37.38			
2474	E5	S	3	0.9363	0.955	0.573		143.254			35.72			
2475	E5	I	1	0.9972	1.017	0.610	0.575	152.572	143.64	7.75	38.05	1.93	35.82	
2476	E5	I	2	0.912	0.930	0.558		139.536			34.80			
2477	E5	I	3	0.9072	0.925	0.555		138.802			34.61			

Table I - 4

In-Vitro Gastrointestinal Method with FeOx Adsorption (IVG-AB)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	std dev	average	3050 As mg/kg
2908	C1	S	1	1.369	1.396	0.838	0.738	209.457	177.43	23.35	1.85	0.21	1.57	11294
2909	C1	S	2	1.067	1.088	0.653		163.251			1.45			
2910	C1	S	3	1.180	1.204	0.722		180.540			1.60			
2911	C1	I	1	1.232	1.257	0.754	0.644	188.496	161.08	23.87	1.67	0.21	1.43	
2912	C1	I	2	0.979	0.999	0.599		149.833			1.33			
2913	C1	I	3	0.947	0.966	0.580		144.906			1.28			
2999	C1	FeOx	1	0.3164	0.316	0.063	0.038	204.316	170.48	30.30	1.81	0.27	1.51	
3000	C1	FeOx	2	0.2284	0.228	0.046		161.253			1.43			
3001	C1	FeOx	3	0.01902	0.019	0.004		145.857			1.29			
2914	C2	S	1	1.403	1.431	0.859	0.799	214.659	199.67	13.31	1.23	0.08	1.14	17456
2915	C2	S	2	1.237	1.262	0.757		189.261			1.08			
2916	C2	S	3	1.275	1.301	0.780		195.075			1.12			
2917	C2	I	1	1.394	1.422	0.853	0.761	213.282	190.13	20.24	1.22	0.12	1.09	
2918	C2	I	2	1.185	1.209	0.725		181.305			1.04			
2919	C2	I	3	1.149	1.172	0.703		175.797			1.01			
3002	C2	FeOx	1	0.03951	0.040	0.008	0.008	215.258	192.18	20.17	1.23	0.12	1.10	
3003	C2	FeOx	2	0.04133	0.041	0.008		183.372			1.05			
3004	C2	FeOx	3	0.042	0.042	0.008		177.913			1.02			
2920	C3	S	1	2.347	2.394	1.436	1.351	359.091	337.67	18.56	2.67	0.14	2.51	13472
2921	C3	S	2	2.134	2.177	1.306		326.502			2.42			
2922	C3	S	3	2.140	2.183	1.310		327.420			2.43			
2923	C3	I	1	2.024	2.064	1.239	1.180	309.672	294.88	13.94	2.30	0.10	2.19	
2924	C3	I	2	1.843	1.880	1.128		281.979			2.09			
2925	C3	I	3	1.915	1.953	1.172		292.995			2.17			
3005	C3	FeOx	1	0.058	0.058	0.012	0.011	312.578	297.71	13.92	2.32	0.10	2.21	
3006	C3	FeOx	2	0.060	0.060	0.012		284.992			2.12			
3007	C3	FeOx	3	0.052	0.052	0.010		295.572			2.19			
2926	C4	S	1	5.652	5.765	3.459	3.215	864.756	803.66	53.06	7.50	0.46	6.97	11525
2927	C4	S	2	5.027	5.128	3.077		769.131			6.67			
2928	C4	S	3	5.079	5.181	3.108		777.087			6.74			

Table I - 4

In-Vitro Gastrointestinal Method with FeOx Adsorption (IVG-AB)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	std dev	average	3050 As mg/kg
2929	C4	I	1	5.221	5.325	3.195	3.099	798.813	774.84	44.73	6.93	0.39	6.72	
2930	C4	I	2	5.245	5.350	3.210		802.485			6.96			
2931	C4	I	3	4.727	4.822	2.893		723.231			6.28			
3008	C4	FeOx	1	0.168	0.168	0.034	0.034	807.213	783.40	46.22	7.00	0.40	6.80	
3009	C4	FeOx	2	0.207	0.207	0.041		812.855			7.05			
3010	C4	FeOx	3	0.138	0.138	0.028		730.121			6.34			
2932	C5	S	1	3.732	3.807	2.284	2.189	570.996	547.13	21.21	9.14	0.34	8.76	6245
2933	C5	S	2	3.467	3.536	2.122		530.451			8.49			
2934	C5	S	3	3.529	3.600	2.160		539.937			8.65			
2935	C5	I	1	3.496	3.566	2.140	2.009	534.888	502.15	30.53	8.57	0.49	8.04	
2936	C5	I	2	3.101	3.163	1.898		474.453			7.60			
2937	C5	I	3	3.249	3.314	1.988		497.097			7.96			
3011	C5	FeOx	1	0.200	0.200	0.040	0.051	544.878	514.89	26.15	8.73	0.42	8.24	
3012	C5	FeOx	2	0.448	0.448	0.090		496.848			7.96			
3013	C5	FeOx	3	0.117	0.117	0.023		502.952			8.05			
2938	S1	S	1	0.858	0.875	0.525	0.487	131.320	121.79	8.35	32.42	2.06	30.07	405
2939	S1	S	2	0.773	0.789	0.473		118.284			29.21			
2940	S1	S	3	0.757	0.772	0.463		115.775			28.59			
2941	S1	I	1	0.718	0.732	0.439	0.413	109.793	103.36	8.21	27.11	2.03	25.52	
2942	S1	I	2	0.615	0.627	0.376		94.110			23.24			
2943	S1	I	3	0.694	0.708	0.425		106.167			26.21			
3014	S1	FeOx	1	0.045	0.045	0.009	0.008	112.053	105.42	8.22	27.67	2.03	26.03	
3015	S1	FeOx	2	0.042	0.042	0.008		96.225			23.76			
3016	S1	FeOx	3	0.037	0.037	0.007		107.994			26.67			
2944	S2	S	1	1.218	1.242	0.745	0.770	186.354	192.40	7.11	41.41	1.58	42.76	450
2945	S2	S	2	1.298	1.324	0.794		198.594			44.13			
2946	S2	S	3	1.217	1.241	0.745		186.201			41.38			
2947	S2	I	1	1.137	1.160	0.696	0.693	173.961	173.25	9.66	38.66	2.15	38.50	
2948	S2	I	2	1.067	1.088	0.653		163.251			36.28			
2949	S2	I	3	1.193	1.217	0.730		182.529			40.56			

Table I - 4

In-Vitro Gastrointestinal Method with FeOx Adsorption (IVG-AB)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	std dev	average	3050 As mg/kg
3017	S2	FeOx	1	0.103	0.103	0.021	0.018	179.091	177.86	9.88	39.80	2.19	39.52	
3018	S2	FeOx	2	0.083	0.083	0.017		167.420			37.20			
3019	S2	FeOx	3	0.091	0.091	0.018		187.056			41.57			
2950	S3	S	1	2.396	2.444	1.466	1.586	366.588	396.53	68.23	31.07	5.78	33.60	1180
2951	S3	S	2	2.277	2.323	1.394		348.381			29.52			
2952	S3	S	3	3.102	3.164	1.898		474.606			40.22			
2953	S3	I	1	2.105	2.147	1.288	1.303	322.065	325.69	11.53	27.29	0.98	27.60	
2954	S3	I	2	2.213	2.257	1.354		338.589			28.69			
2955	S3	I	3	2.068	2.109	1.266		316.404			26.81			
3020	S3	FeOx	1	0.179	0.179	0.036	0.028	331.010	332.62	9.72	28.05	0.82	28.19	
3021	S3	FeOx	2	0.089	0.089	0.018		343.045			29.07			
3022	S3	FeOx	3	0.148	0.148	0.030		323.809			27.44			
2956	S4	S	1	10.050	10.251	6.151	5.448	1537.650	1362.11	155.10	30.62	3.09	27.12	5022
2957	S4	S	2	8.128	8.291	4.974		1243.584			24.76			
2958	S4	S	3	8.53	8.701	5.220		1305.090			25.99			
2959	S4	I	1	9.189	9.373	5.624	4.856	1405.917	1213.90	167.29	28.00	3.33	24.17	
2960	S4	I	2	7.187	7.331	4.398		1099.611			21.90			
2961	S4	I	3	7.426	7.575	4.545		1136.178			22.62			
3023	S4	FeOx	1	0.450	0.450	0.090	0.076	1428.412	1232.85	170.27	28.44	3.39	24.55	
3024	S4	FeOx	2	0.358	0.358	0.072		1117.501			22.25			
3025	S4	FeOx	3	0.329	0.329	0.066		1152.638			22.95			
2962	S5	S	1	6.318	6.444	3.867	3.568	966.654	891.94	66.17	20.79	1.42	19.18	4650
2963	S5	S	2	5.676	5.790	3.474		868.428			18.68			
2964	S5	S	3	5.495	5.605	3.363		840.735			18.08			
2965	S5	I	1	5.531	5.642	3.385	3.172	846.243	793.08	75.19	18.20	9.91	17.06	
2966	S5	I	2		0.000	0.000		0.000			0.00			
2967	S5	I	3	4.836	4.933	2.960		739.908			15.91			
3026	S5	FeOx	1	0.331	0.331	0.066	#VALUE!	862.778	809.83	74.88	18.55	1.61	17.42	
3027	S5	FeOx	2	no daata	#VALUE!	#VALUE!		#VALUE!			#VALUE!			
3028	S5	FeOx	3	0.340	0.340	0.068		756.883			16.28			

Table I - 4

In-Vitro Gastrointestinal Method with FeOx Adsorption (IVG-AB)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	std dev	average	3050 As mg/kg
2968	E1	S	1	0.197	0.201	0.121	0.119	30.126	29.84	1.75	9.10	0.53	9.02	331
2969	E1	S	2	0.183	0.186	0.112		27.968			8.45			
2970	E1	S	3	0.205	0.210	0.126		31.426			9.49			
2971	E1	I	1	0.245	0.249	0.150	0.154	37.409	38.41	9.35	11.30	2.82	11.61	
2972	E1	I	2	0.315	0.322	0.193		48.226			14.57			
2973	E1	I	3	0.194	0.197	0.118		29.606			8.94			
3029	E1	FeOx	1	0.134	0.134	0.027	0.015	44.114	42.22	8.44	13.33	2.55	12.75	
3030	E1	FeOx	2	0.026	0.026	0.005		49.547			14.97			
3031	E1	FeOx	3	0.068	0.068	0.014		32.993			9.97			
2974	E2	S	1	~~1.049~~	~~1.070~~	0.642	0.400	no use	69.79	1.96	#VALUE!	0.84	29.95	233
2975	E2	S	2	0.447	0.456	0.274		68.406			29.36			
2976	E2	S	3	0.465	0.475	0.285		71.176			30.55			
2977	E2	I	1	0.455	0.464	0.278	0.253	69.584	63.15	5.62	29.86	2.41	27.10	
2978	E2	I	2	0.397	0.405	0.243		60.680			26.04			
2979	E2	I	3	0.387	0.395	0.237		59.180			25.40			
3032	E2	FeOx	1	0.062	0.062	0.012	0.006	72.664	64.76	6.89	31.19	2.96	27.79	
3033	E2	FeOx	2	0.019	0.019	0.004		61.626			26.45			
3034	E2	FeOx	3	0.016	0.016	0.003		59.997			25.75			
2980	E3	S	1	2.071	2.112	1.267	1.188	316.863	296.92	17.70	39.61	2.21	37.12	800
2981	E3	S	2	1.901	1.939	1.163		290.853			36.36			
2982	E3	S	3	1.85	1.887	1.132		283.050			35.38			
2983	E3	I	1	1.898	1.936	1.162	1.030	290.394	257.50	28.49	36.30	3.56	32.19	
2984	E3	I	2	1.578	1.610	0.966		241.434			30.18			
2985	E3	I	3	1.573	1.604	0.963		240.669			30.08			
3035	E3	FeOx	1	0.149	0.149	0.030	0.025	297.819	263.78	29.61	37.23	3.70	32.97	
3036	E3	FeOx	2	0.050	0.050	0.010		243.937			30.49			
3037	E3	FeOx	3	0.179	0.179	0.036		249.594			31.20			
2986	E4	S	1	0.8469	0.864	0.518	0.635	129.576	127.65	2.73	8.86	0.19	8.73	1463
2987	E4	S	2	1.446	1.475	0.885		no use			#VALUE!			
2988	E4	S	3	0.8217	0.838	0.503		125.720			8.59			

Table I - 4
In-Vitro Gastrointestinal Method with FeOx Adsorption (IVG-AB)

Sample No.	Sample ID	GI Phase	Rep No.	As mg/L	Corrected As (mg/L)	As mg	Ave As mg	As mg/kg	Ave As mg/kg	Std Dev	Bioavailable As (%)	std dev	average	3050 As mg/kg
2989	E4	I	1	0.6989	0.713	0.428	0.454	106.932	113.55	5.73	7.31	0.39	7.76	
2990	E4	I	2	0.7628	0.778	0.467		116.708			7.98			
2991	E4	I	3	0.7647	0.780	0.468		116.999			8.00			
3038	E4	FeOx	1	0.336	0.336	0.067	0.033	123.717	121.84	1.64	8.46	0.11	8.33	
3039	E4	FeOx	2	0.089	0.089	0.018		121.158			8.28			
3040	E4	FeOx	3	0.073	0.073	0.015		120.658			8.25			
2992	E5	S	1	1.108	1.130	0.678	0.634	169.524	158.47	9.61	42.28	2.40	39.52	401
2993	E5	S	2	0.9942	1.014	0.608		152.113			37.93			
2994	E5	S	3	1.005	1.025	0.615		153.765			38.35			
2995	E5	I	1		0.000	0.000	0.529	no data	132.35	8.07	#VALUE!	2.01	33.00	
2996	E5	I	2	0.9023	0.920	0.552		138.052			34.43			
2997	E5	I	3	0.8277	0.844	0.507		126.638			31.58			
3041	E5	FeOx	1	0.067	0.067	0.013	0.014	no data	135.97	6.72	#VALUE!	1.68	33.91	
3042	E5	FeOx	2	0.053	0.053	0.011		140.722			35.09			
3043	E5	FeOx	3	0.092	0.092	0.018		131.221			32.72			

Table I - 5
Arsenic Bioavailability by Experimental Method; Summary Statistics

Sample ID	Rep	GI-IV		GI-IV-AB		PBET		In-Vivo
		Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	individual (%)
C1	1	1.75	1.26	1.85	1.81	0.78	0.90	0.60
C1	2	1.43	1.26	1.45	1.43	0.57	0.80	5.51
C1	3	1.56	1.21	1.60	1.29	0.68	0.96	3.13
C1	4	...	...	...	...	...	...	1.64
C1	5	...	...	...	...	...	...	2.68
C2	1	0.96	0.98	1.23	1.23	0.69	0.74	4.62
C2	2	0.99	0.94	1.08	1.05	0.43	1.19	3.72
C2	3	0.98	1.01	1.12	1.02	0.41	0.53	3.87
C2	4	...	...	...	...	...	...	-0.15
C2	5	...	...	...	...	...	...	4.47
C3	1	2.29	2.16	2.67	2.32	1.23	1.35	10.43
C3	2	2.12	2.36	2.42	2.12	0.90	1.20	10.28
C3	3	2.20	2.16	2.43	2.19	0.69	1.13	7.00
C3	4	...	...	...	...	...	...	5.96
C3	5	...	...	...	...	...	...	8.04
C4	1	5.95	5.89	7.50	7.00	1.90	2.03	19.36
C4	2	6.02	5.84	6.67	7.05	1.74	2.00	19.36
C4	3	5.94	5.57	6.74	6.34	1.94	2.01	26.81
C4	4	...	...	...	...	...	...	23.83
C4	5	...	...	...	...	...	...	20.85
C5	1	7.64	7.49	9.14	8.73	3.35	3.00	38.73
C5	2	7.48	7.40	8.49	7.96	2.15	2.19	38.73
C5	3	7.55	7.21	8.65	8.05	4.16	2.05	23.83
C5	4	...	...	...	...	...	...	23.83
C5	5	...	...	...	...	...	...	29.79

Table I - 5
Arsenic Bioavailability by Experimental Method; Summary Statistics

Sample		GI-IV		GI-IV-AB		PBET		In-Vivo
ID	Rep	Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	individual (%)
S1	1	27.50	24.45	32.42	27.67	42.59	16.58	...
S1	2	24.85	21.52	29.21	23.76	35.13	21.00	...
S1	3	28.03	21.47	28.59	26.67	40.60	6.98	...
S1	4	...	...	...	...	...	...	...
S1	5	...	...	...	...	...	...	...
S2	1	37.60	28.67	41.41	39.80	42.57	15.01	...
S2	2	35.56	36.01	44.13	37.20	36.52	15.01	...
S2	3	42.67	36.41	41.38	41.57	52.63	21.02	...
S2	4	...	...	...	...	...	...	...
S2	5	...	...	...	...	...	...	...
S3	1	33.85	33.06	31.07	28.05	4.03	16.77	29.79
S3	2	28.32	26.00	29.52	29.07	24.00	16.48	36.42
S3	3	31.17	26.71	40.22	27.44	19.54	16.34	16.24
S3	4	...	...	...	...	...	...	15.49
S3	5	...	...	...	...	...	...	45.58
S4	1	31.23	29.33	30.62	28.44	17.21	11.30	38.73
S4	2	22.74	21.29	24.76	22.25	9.61	8.09	38.73
S4	3	22.89	20.90	25.99	22.95	15.29	11.19	23.83
S4	4	...	...	...	...	...	...	23.83
S4	5	...	...	...	...	...	...	29.79
S5	1	22.06	21.03	20.79	18.55	12.28	9.54	22.05
S5	2	15.64	11.55	18.68	...	10.54	9.09	12.81
S5	3	15.72	14.22	18.08	16.28	12.27	9.12	16.68
S5	4	...	...	...	...	...	...	14.75
S5	5	...	...	...	...	...	...	15.94

Table I - 5
Arsenic Bioavailability by Experimental Method; Summary Statistics

Sample		GI-IV		GI-IV-AB		PBET		In-Vivo
ID	Rep	Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	Stomach As (%)	Intestine As (%)	individual (%)
E1	1	10.71	9.38	9.10	13.33	4.17	5.53	12.96
E1	2	8.40	8.02	8.45	14.97	5.31	12.10	6.26
E1	3	9.53	8.30	9.49	9.97	5.82	5.06	1.12
E1	4	...	...	...	...	...	...	4.36
E1	5	...	...	...	...	...	...	6.13
E2	1	41.75	31.05	...	31.19	21.71	14.70	55.64
E2	2	30.49	25.83	29.36	26.45	16.66	27.82	37.09
E2	3	33.52	25.25	30.55	25.75	21.91	17.16	45.13
E2	4	...	...	...	...	...	...	33.31
E2	5	...	...	...	...	...	...	...
E3	1	32.38	27.79	39.61	37.23	24.57	10.66	29.05
E3	2	32.51	27.90	36.36	30.49	26.62	8.45	34.19
E3	3	32.63	29.20	35.38	31.20	29.16	11.12	25.47
E3	4	...	...	...	...	...	...	33.52
E3	5	...	...	...	...	...	...	23.46
E4	1	8.77	7.10	8.86	8.46	16.56	8.98	15.64
E4	2	8.33	7.29	...	8.28	11.61	7.89	18.77
E4	3	8.46	7.63	8.59	8.25	17.30	8.86	16.53
E4	4	...	...	...	...	...	...	23.68
E4	5	...	...	...	...	...	...	...
E5	1	41.93	38.05	42.28	...	34.72	19.40	36.64
E5	2	37.38	34.80	37.93	35.09	32.61	20.11	32.62
E5	3	35.72	34.61	38.35	32.72	44.97	14.33	37.98
E5	4	...	...	...	...	...	...	37.54
E5	5	...	...	...	...	...	...	37.54

Appendix II

In-Vivo Data

Table II - 1
Feeding Trial 1: Soils C4 and S4

		Day -1			Day 3			Day 7		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
CT-OSU1	221	5.091	1500	7.637	6.429	1380	8.872	12.061	1100	13.267
CT-OSU1	230	5.95	860	5.117	2.836	2140	6.069	6.545	2500	16.363
CT-OSU1	243	11.19	720	8.057	10.104	1140	11.519	10.688	1220	13.039
R1-OSU1	222	4.866	2360	11.484	38.565	1040	40.108	42.619	1200	51.143
R1-OSU1	227	5.521	1160	6.404	12.338	3220	39.728	13.698	3760	51.504
R1-OSU1	235	5.871	820	4.814	28.397	1140	32.373	33.265	1400	46.571
R1-OSU1	249	6.647	840	5.583	18.442	1900	35.040	16.125	2850	45.956
R1-OSU1	251	6.543	1460	9.553	34.794	820	28.531	31.511	1380	43.485
R2-OSU1	216	14.95	680	10.166	84.383	900	75.945	76.892	1400	107.649
R2-OSU1	218	4.017	2460	9.882	34.643	3020	104.622	52.743	2440	128.693
R2-OSU1	225	8.925	1020	9.104	59.566	1540	91.732	77.551	1300	100.816
R2-OSU1	235	5.247	1260	6.611	96.275	1080	103.977	137.907	920	126.874
R2-OSU1	240	15.59	760	11.848	244.409	1080	263.962	111.746	1120	125.156
R3-OSU1	205	8.728	1460	12.743	135.983	1380	187.657	202.313	1750	354.048
R3-OSU1	208	8.197	1200	9.836	153.783	1980	304.490	288.957	1200	346.748
R3-OSU1	215	16.05	760	12.198	81.068	3580	290.223	214.822	1600	343.715
R3-OSU1	224	15.62	1460	22.805	585.417	1560	913.251	629.925	740	466.145
R3-OSU1	252	9.059	1200	10.871	173.946	1800	313.103	202.713	1800	364.883
C1-OSU1	201	6.106	740	4.518	18.443	1420	26.189	42.607	1240	52.833
C1-OSU1	206	7.337	680	4.989	17.621	1320	23.260	28.208	1500	42.312
C1-OSU1	217	6.668	1560	10.402	42.91	600	25.746	33.326	880	29.327
C1-OSU1	223	9.813	980	9.617	8.126	2560	20.803	3.591	7740	27.794
C1-OSU1	238	14.4	640	9.216	105.752	500	52.876	4.909	**	
C2-OSU1	214	14.33	750	10.748	139.762	520	72.676	50.451	1240	62.559
C2-OSU1	233	15.88	480	7.622	61.879	820	50.741	52.931	1220	64.576
C2-OSU1	241	14.25	700	9.975	54.224	880	47.717	100.274	860	86.236
C2-OSU1	246	8.262	800	6.610	32.062	1560	50.017	64.015	1350	86.420
C2-OSU1	248	3.133	3020	9.462	34.124	1800	61.423	29.816	2680	79.907

Table II - 1
Feeding Trial 1: Soils C4 and S4

		Day -1			Day 3			Day 7		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
C3-OSU1	202	7.885	1200	9.462	78.478	1140	89.465	137.516	1100	151.268
C3-OSU1	203	11.65	800	9.320	80.447	1020	82.056	170.733	1040	177.562
C3-OSU1	229	9.05	820	7.421	94.201	680	64.057	145.041	900	130.537
C3-OSU1	231	2.433	3900	9.489	37.929	1940	73.582	98.499	1680	165.478
C3-OSU1	250	3.49	2440	8.516	no samp	3380		46.927	3020	141.720
S1-OSU1	204	3.044	2260	6.879	22.788	1180	26.890	37.679	1400	52.751
S1-OSU1	228	20.68	460	9.513	31.137	1060	33.005	72.345	640	46.301
S1-OSU1	237	11.92	780	9.298	68.156	1040	70.882	60.013	1260	75.616
S1-OSU1	239	12.91	1020	13.168	29.533	1260	37.212	42.762	1080	46.183
S1-OSU1	253	17.92	760	13.619	3.757	4460	16.756	16.883	3760	63.480
S2-OSU1	207	19.9	720	14.328	86.473	1260	108.956	71.435	1600	114.296
S2-OSU1	212	6.609	1720	11.367	48.255	1700	82.034	88.989	1560	138.823
S2-OSU1	213	11.23	740	8.310	71.028	980	69.607	44.836	1320	59.184
S2-OSU1	220	22.31	380	8.478	78.733	680	53.538	112.912	860	97.104
S2-OSU1	244	8.575	940	8.061	85.53	1000	85.530	123.615	1020	126.087
S3-OSU1	211	19.74	420	8.291	170.095	640	108.861	85.548	1280	109.501
S3-OSU1	219	10.9	1240	13.516	52.025	2800	145.670	91.347	4600	420.196
S3-OSU1	232	1.504	4000	6.016	99.239	1940	192.524	88.901	2540	225.809
S3-OSU1	234	0.657	10980	7.214	23.84	6260	149.238	20.059	5680	113.935
S3-OSU1	242	2.528	2520	6.371	14.434	4940	71.304	18.115	6600	119.559

**Pig 238 had waterer leak. Leak ran into pig 223's pen.
3 buckets: 10,000 = light
11500 = clear
5640 = medium
took sample from medium bucket

Table II - 1
Feeding Trial 1: Soils C4 and S4

		Day 11			Day 15		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
CT-OSU1	221	5.962	2820	16.813	6.712	3240	21.747
CT-OSU1	230	22.14	2040	45.166	7.281	2820	20.532
CT-OSU1	243	6.596	700	4.617	13.967	1420	19.833
R1-OSU1	222	44.1	1280	56.448	32.94	1520	50.069
R1-OSU1	227	15.488	4820	74.652	15.492	5400	83.657
R1-OSU1	235	24.165	2600	62.829	59.246	1100	65.171
R1-OSU1	249	4.539	11000	49.929	17.483	4480	78.324
R1-OSU1	251	27.654	1740	48.118	51.507	2580	132.888
R2-OSU1	216	70.531	1500	105.797	23.592	4500	106.164
R2-OSU1	218	46.582	2480	115.523	19.38	3780	73.256
R2-OSU1	225	122.578	1180	144.642	40.582	1340	54.380
R2-OSU1	235	198.452	850	168.684	130.95	1200	157.140
R2-OSU1	240	102.74	1330	136.644	152.359	1680	255.963
R3-OSU1	205	326.355	1240	404.680	262.199	2220	582.082
R3-OSU1	208	112.586	3100	349.017	120.086	3620	434.711
R3-OSU1	215	98.962	2880	285.011	39.578	12260	485.226
R3-OSU1	224	261.326	740	193.381	317.732	1820	578.272
R3-OSU1	252	424.316	1240	526.152	300.397	1440	432.572
C1-OSU1	201	24.895	1320	32.861	146.119	1060	154.886
C1-OSU1	206	34.389	1400	48.145	24.975	2620	65.435
C1-OSU1	217	63.57	580	36.871	77.302	960	74.210
C1-OSU1	223	9.812	3820	37.482	6.919	6700	46.357
C1-OSU1	238	14.921	1920	28.648	23.499	2880	67.677
C2-OSU1	214	100.966	750	75.725	67.078	1700	114.033
C2-OSU1	233	47.288	1460	69.040	98.88	1160	114.701
C2-OSU1	241	115.292	880	101.457	175.884	820	144.225
C2-OSU1	246	75.981	1160	88.138	85.874	1400	120.224
C2-OSU1	248	34.54	2260	78.060	34.671	3300	114.414

Table II - 1
Feeding Trial 1: Soils C4 and S4

		Day 11			Day 15		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
C3-OSU1	202	172.032	900	154.829	394.798	660	260.567
C3-OSU1	203	131.478	1400	184.069	131	2000	262.000
C3-OSU1	229	155.64	1020	158.753	96.599	1640	158.422
C3-OSU1	231	59.642	2200	131.212	73.985	2720	201.239
C3-OSU1	250	24.994	4980	124.470	64.277	3000	192.831
S1-OSU1	204	43.435	1980	86.001	44.488	2200	97.874
S1-OSU1	228	150.903	650	98.087	83.299	860	71.637
S1-OSU1	237	86.903	960	83.427	20.897	4060	84.842
S1-OSU1	239	57.81	1360	78.622	84.907	1020	86.605
S1-OSU1	253	30.552	2840	86.768	36.79	2580	94.918
S2-OSU1	207	79.24	1940	153.726	96.961	1940	188.104
S2-OSU1	212	50.7	2760	139.932	177.481	1100	195.229
S2-OSU1	213	64.193	1660	106.560	108.015	1500	162.023
S2-OSU1	220	109.664	840	92.118	140.193	860	120.566
S2-OSU1	244	152.487	880	134.189	67.487	1640	110.679
S3-OSU1	211	28.463	2540	72.296	53.723	1740	93.478
S3-OSU1	219	68.33	3600	245.988	77.118	4780	368.624
S3-OSU1	232	46.353	2960	137.205	108.812	3100	337.317
S3-OSU1	234	71.871	4720	339.231	38.74	6780	262.657
S3-OSU1	242	106.495	2320	247.068	86.62	3100	268.522

**Pig 238 had watere
3 buckets: 10,000 = li
11500 =
5640 =
took sample from med

Table II - 2
Feeding Trial 1: Soils C4 and S4
Kidney Digests (Second Set)
Sample Date 3/24/97 and 3/26/97 Analysis Date 8/12/97 and 8/14/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
1953	CT	221	16	K	1.125
1954	CT	230	16	K	1.156
1955	CT	243	16	K	1.036
1956	R1	222	16	K	2.733
1957	R1	227	16	K	1.415
1958	R1	235	16	K	1.108
1959	R1	249	16	K	1.151
1960	R1	251	16	K	1.861
1961	R2	216	16	K	3.779
1962	R2	218	16	K	2.453
1963	R2	225	16	K	2.292
1964	R2	236	16	K	2.208
1965	R2	240	16	K	1.522
1966	R3	205	16	K	5.453
1967	R3	208	16	K	4.654
1968	R3	215	16	K	3.11
1969	R3	224	16	K	6.228
1970	R3	252	16	K	3.386
1971	C1	201	16	K	2.548
1972	C1	206	16	K	1.393
1973	C1	217	16	K	1.637
1974	C1	223	16	K	1.639
1975	C1	238	16	K	1.911
1976	C2	214	16	K	1.62
1977	C2	233	16	K	2.781
1978	C2	241	16	K	3.471
1979	C2	246	16	K	5.158
1980	C2	248	16	K	3.834
1981	C3	202	16	K	5.315
1982	C3	203	16	K	6.172
1983	C3	229	16	K	4.764
1984	C3	231	16	K	5.05
1985	C3	250	16	K	4.974
1986	S1	204	16	K	2.777
1987	S1	228	16	K	2.641
1988	S1	237	16	K	3.392
1989	S1	239	16	K	2.939
1990	S1	253	16	K	3.221
1991	S2	207	16	K	4.592
1992	S2	212	16	K	4.188
1993	S2	213	16	K	3.461
1994	S2	220	16	K	3.283
1995	S2	244	16	K	4.616

Feeding Trial 1: Soils C4 and S4
Kidney Digests (Second Set)
Sample Date 3/24/97 and 3/26/97 Analysis Date 8/12/97 and 8/14/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
1996	S3	211	16	K	4.318
1997	S3	219	16	K	7.051
1998	S3	232	16	K	7.346
1999	S3	234	16	K	5.603
2000	S3	242	16	K	6.392
2001	R2	218L	16	K	4.514
2002	C3	2203	16	K	6.343
2003	R1	2222	16	K	4.101
2004	P. Blank				*
2005	P. Blank				*
2006	P. Blank				*

*Because the digestion blanks were so high in nitric acid (too oxidizing) they could not be read by hydride generation without dilution by 50%. This would have resulted in losing the arsenic value by dilution, therefore no arsenic concentration could be measured. If "trace metal" grade nitric acid was used in the digest, there should be no appreciable arsenic contamination.

Table II - 3
Feeding Trial 1: Soils C4 and S4
Liver Digests
Sample Date 3/24/97 and 3/26/97 Analysis Date 7/10/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
1534	CT	221	16	Li	0.382
1535	CT	230	16	Li	1.394
1536	CT	243	16	Li	1.031
1537	R1	222	16	Li	0.783
1538	R1	227	16	Li	0.659
1539	R1	235	16	Li	0.467
1540	R1	249	16	Li	0.604
1541	R1	251	16	Li	< 0.3
1542	R2	216	16	Li	0.565
1543	R2	218	16	Li	< 0.3
1544	R2	225	16	Li	0.393
1545	R2	236	16	Li	< 0.3
1546	R2	240	16	Li	< 0.3
1547	R3	205	16	Li	0.886
1548	R3	208	16	Li	0.644
1549	R3	215	16	Li	< 0.3
1550	R3	224	16	Li	0.935
1551	R3	252	16	Li	0.788
1552	C1	201	16	Li	0.417
1553	C1	206	16	Li	< 0.3
1554	C1	217	16	Li	< 0.3
1555	C1	223	16	Li	0.446
1556	C1	238	16	Li	0.451
1557	C2	214	16	Li	0.385
1558	C2	233	16	Li	0.347
1559	C2	241	16	Li	0.778
1560	C2	246	16	Li	0.544
1561	C2	248	16	Li	0.785
1562	C3	202	16	Li	0.841
1563	C3	203	16	Li	1.036
1564	C3	229	16	Li	0.764
1565	C3	231	16	Li	1.051
1566	C3	250	16	Li	0.826
1567	S1	204	16	Li	0.717
1568	S1	228	16	Li	0.711
1569	S1	237	16	Li	0.709
1570	S1	239	16	Li	0.864
1571	S1	253	16	Li	0.653
1572	S2	207	16	Li	0.897
1573	S2	212	16	Li	0.786
1574	S2	213	16	Li	0.315
1575	S2	220	16	Li	< 0.3
1576	S2	244	16	Li	< 0.3

Table II - 3
Feeding Trial 1: Soils C4 and S4
Liver Digests
Sample Date 3/24/97 and 3/26/97 Analysis Date 7/10/97

OSU Sample No.	UMC Sample Description				As
	Sample ID	Pig No.	Day No.	Tissue	ug/l
1577	S3	211	16	Li	< 0.3
1578	S3	219	16	Li	< 0.3
1579	S3	232	16	Li	< 0.3
1580	S3	234	16	Li	0.376
1581	S3	242	16	Li	0.301
1582	C3	2203	16	Li	0.664
1583	R3	2205	16	Li	0.931
1584	S3	2234	16	Li	< 0.3
1585	P Blank				*
1586	P Blank				*
1587	P Blank				*

*Because the digestion blanks were so high in nitric acid (too oxidizing) they could not be read by hydride generation without dilution by 50%. This would have resulted in losing the arsenic value by dilution, therefore no arsenic concentration could be measured. If "trace metal" grade nitric acid was used in the digest, there shou be no appreciable arsenic contamination.

Table II - 4

Feeding Trial 1: Soils C4 and S4

Bile Digests

Sample Date 3/24/97 and 3/26/97 Analysis Date 10/9/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2534	CT	221	16	K	< 0.3
2535	CT	230	16	K	< 0.3
2536	CT	243	16	K	< 0.3
2537	R1	222	16	K	0.277
2538	R1	227	16	K	0.421
2539	R1	235	16	K	0.376
2540	R1	249	16	K	0.475
2541	R1	251	16	K	2.342
2542	R2	216	16	K	< 0.3
2543	R2	218	16	K	0.164
2544	R2	225	16	K	< 0.3
2545	R2	236	16	K	0.014
2546	R2	240	16	K	0.975
2547	R3	205	16	K	< 0.3
2548	R3	208	16	K	< 0.3
2549	R3	215	16	K	< 0.3
2550	R3	224	16	K	5.14
2551	R3	252	16	K	0.188
2552	C1	201	16	K	0.14
2553	C1	206	16	K	0.922
2554	C1	217	16	K	< 0.3
2555	C1	223	16	K	0.318
2556	C1	238	16	K	0.204
2557	C2	214	16	K	< 0.3
2558	C2	233	16	K	< 0.3
2559	C2	241	16	K	2.071
2560	C2	246	16	K	0.775
2561	C2	248	16	K	0.244
2562	C3	202	16	K	1.736
2563	C3	203	16	K	0.377
2564	C3	229	16	K	0.436
2565	C3	231	16	K	0.347
2566	C3	250	16	K	1.434

Table II - 4

Feeding Trial 1: Soils C4 and S4

Bile Digests

Sample Date 3/24/97 and 3/26/97 Analysis Date 10/9/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2567	S1	204	16	K	< 0.3
2568	S1	228	16	K	1.344
2569	S1	237	16	K	< 0.3
2570	S1	239	16	K	4.362
2571	S1	253	16	K	1.401
2572	S2	207	16	K	1.155
2573	S2	212	16	K	0.946
2574	S2	213	16	K	0.624
2575	S2	220	16	K	0.873
2576	S2	244	16	K	0.71
2577	S3	211	16	K	0.637
2578	S3	219	16	K	0.386
2579	S3	232	16	K	0.301
2580	S3	234	16	K	0.796
2581	S3	242	16	K	0.617
2582	S3	2234	16	K	0.699
2583	C2	2248	16	K	0.631

Table II - 5
Analysis Date 3/27/97

OSU Sample No.	UMC Sample Description				As ug/L
	Sample ID	Date Collected	Matrix	Remarks	
769	OSU1-01	3/7/97	water	WK 1 - pig 205 pen	0.3991
770	OSU1-02	3/7/97	water	WK 1- pig 204 pen	0.6569
771	OSU1-04	3/24/97	water	WK 2 - pig 242 pen	0.7071
772	OSU1-05	3/24/97	water	WK 2 - pig 203 pen	0.9702

Analysis Date 5/12/97

OSU Sample No.	UMC Sample Description				As ug/g
	Sample ID	Date Collected	Matrix	Remarks	
1014	OSU1-03	3/7/97	feed	S-2 feed, from UMC Feedmill	0.253

Feed sample prepared by first grinding to powder, then 1.00 g samples digested using USEPA SW-846, Method 3050

Table II - 6
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5

		Day 0			Day 4			Day 8		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
CT	302	2.228	1970	4.389	4.07	1720	7.000	3.865	1920	7.421
CT	338	3.782	1060	4.009	5.274	1630	8.597	2.103	3160	6.645
CT	341	10.786	480	5.177	12.621	490	6.184	7.665	700	5.366
C1	306	12.334	470	5.797	38.179	220	8.399	13.847	620	8.585
C1	317	9.523	620	5.904	22.193	580	12.872	12.83	1220	15.653
C1	324	8.622	630	5.432	71.905	200	14.381	21.75	620	13.485
C1	327	4.293	1600	6.869	9.168	1220	11.185	7.695	2020	15.544
C1	348	18.915	290	5.485	29.445	180	5.300	21.154	300	6.346
C2	318	4.049	1600	6.478	8.945	1180	10.555	9.366	2040	19.107
C2	319	1.29	3700	4.773	16.176	1080	17.470	10.386	1480	15.371
C2	325	7.553	640	4.834	22.322	780	17.411	9.27	2120	19.652
C2	337	0.3	7900	2.370	18.066	500	9.033	3.014	1200	3.617
C2	339	8.287	350	2.900	18.667	770	14.374	10.391	1700	17.665
C3	309	4.229	1740	7.358	8.983	2800	25.152	17.061	1300	22.179
C3	313	2.308	700	1.616	11.486	1640	18.837	11.571	1740	20.134
C3	322	9.345	520	4.859	24.741	730	18.061	26.254	680	17.853
C3	336	7.253	800	5.802	25.36	840	21.302	7.005	2020	14.150
C3	350	9.878	560	5.532	16.946	600	10.168	29.755	880	26.184
C5	303	1.194	880	1.051	4.274	3000	12.822	12.143	1500	18.215
C5	315	1.033	7480	7.727	17.381	1060	18.424	19.416	760	14.756
C5	328	15.369	450	6.916	28.898	420	12.137	20.666	950	19.633
C5	334	0.3	6560	1.968	35.107	460	16.149	14.133	530	7.490
C5	346	15.811	660	10.435	23.738	640	15.192	21.471	1080	23.189
S1	307	4.531	1670	7.567	6.993	840	5.874	5.349	1180	6.312
S1	312	1.238	1210	1.498	18.783	340	6.386	5.638	940	5.300
S1	326	10.593	520	5.508	17.412	280	4.875	20.563	400	8.225
S1	333	13.198	450	5.939	17.135	300	5.141	16.421	200	3.284
S1	335	7.942	740	5.877	4.928	1410	6.948	9.508	600	5.705

Table II - 6
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5

		Day 0			Day 4			Day 8		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
S2	301	16.304	440	7.174	17.683	740	13.085	14.191	640	9.082
S2	304	9.009	790	7.117	12.751	500	6.376	7.179	1580	11.343
S2	308	6.594	600	3.956	9.548	680	6.493	6.11	1260	7.699
S2	311	2.579	1560	4.023	2.398	3060	7.338	2.081	no volume	
S2	343	1.19	2060	2.451	3.3	1960	6.468	1.511	2560	3.868
S3	316	4.192	1330	5.575	3.58	3200	11.456	1.601	9660	15.466
S3	329	4.888	590	2.884	10.393	980	10.185		no sample	
S3	331	3.453	1360	4.696	4.156	1700	7.065	3.708	1880	6.971
S3	340	4.854	1120	5.436	9.111	870	7.927	5.41	1980	10.712
S3	345	8.978	490	4.399	22.151	520	11.519	10.626	1560	16.577
S5	305	9.859	700	6.901	21.577	1020	22.009	23.989	1000	23.989
S5	320	8.227	760	6.253	7.059	1880	13.271	9.915	1500	14.873
S5	342	5.35	930	4.976	5.904	1600	9.446	9.183	1400	12.856
S5	344	2.597	360	0.935	66.09	220	14.540	38.467	380	14.617
S5	349	9.15	330	3.020	34.998	460	16.099	25.275	740	18.704

Table II - 6
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5

		Day 12			Day 15		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
CT	302	6.118	1630	9.972	3.88	2760	10.709
CT	338	4.439	2320	10.298	1.643	4320	7.098
CT	341	15.597	540	8.422	12.404	610	7.566
C1	306	7.866	680	5.349	17.086	840	14.352
C1	317	10.278	1420	14.595	10.389	2080	21.609
C1	324	18.423	620	11.422	10.561	1400	14.785
C1	327	4.517	1800	8.131	2.891	2560	7.401
C1	348	31.934	560	17.883	77.725	1080	83.943
C2	318	8.538	2280	19.467	8.784	2380	20.906
C2	319	7.712	2220	17.121	12.605	1640	20.672
C2	325	8.815	1980	17.454	3.819	4400	16.804
C2	337	4.975	2360	11.741	2.645	3220	8.517
C2	339	17.612	1240	21.839	9.173	2040	18.713
C3	309	27.537	1730	47.639	7.055	3540	24.975
C3	313	52.463	670	35.150	27.228	1530	41.659
C3	322	19.384	940	18.221	14.359	2580	37.046
C3	336	15.074	1440	21.707	12.486	2400	29.966
C3	350	36.488	660	24.082	23.629	1240	29.300
C5	303	19.455	1220	23.735	19.87	5200	103.324
C5	315	16.827	1900	31.971	93.029	1380	128.380
C5	328	19.126	1960	37.487	20.365	2700	54.986
C5	334	10.339	1040	10.753	39.205	3800	148.979
C5	346	36.079	840	30.306	14.066	2020	28.413
S1	307	5.24	2200	11.528	3.499	1560	5.458
S1	312	7.547	1040	7.849	8.834	740	6.537
S1	326	6.232	1160	7.229	23.039	320	7.372
S1	333	28.69	260	7.459	19.438	600	11.663
S1	335	7.638	1380	10.540	4.391	2640	11.592

Table II - 6
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5

		Day 12			Day 15		
Sample ID	Pig No.	As, ug/L	Urine Vol, ml	As, ug	As, ug/L	Urine Vol, ml	As, ug
S2	301	5.138	720	3.699	13.296	820	10.903
S2	304	11.839	1460	17.285	5.62	1520	8.542
S2	308	7.238	1240	8.975	7.808	1260	9.838
S2	311	5.418	1600	8.669	4.588	2240	10.277
S2	343	1.963	3500	6.871	1.719	6360	10.933
S3	316	3.027	2600	7.870	4.679	3800	17.780
S3	329	15.734	900	14.161	20.962	890	18.656
S3	331	6.434	1620	10.423	5.713	3020	17.253
S3	340	7.41	1450	10.745	14.002	820	11.482
S3	345	16.153	880	14.215	22.256	860	19.140
S5	305	21.16	1360	28.778	19.694	900	17.725
S5	320	7.323	2200	16.111	8.564	2500	21.410
S5	342	15.051	2000	30.102	12.796	1360	17.403
S5	344	46.913	530	24.864	12.437	1320	16.417
S5	349	17.992	960	17.272	28.312	820	23.216

Table II - 7

Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5

Kidney Digests (Second Set)

Sample Date 5/27/97 Analysis Date 9/3/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2019	CT	302	16	K	0.964
2020	CT	338	16	K	0.872
2021	CT	341	16	K	0.517
2022	C1	306	16	K	1.192
2023	C1	317	16	K	1.356
2024	C1	324	16	K	0.967
2025	C1	327	16	K	1.147
2026	C1	348	16	K	1.313
2027	C2	318	16	K	1.376
2028	C2	319	16	K	1.517
2029	C2	325	16	K	1.45
2030	C2	337	16	K	1.424
2031	C2	339	16	K	2.304
2032	C3	309	16	K	2.474
2033	C3	313	16	K	2.999
2034	C3	322	16	K	2.395
2035	C3	336	16	K	1.8
2036	C3	350	16	K	2.078
2037	C5	303	16	K	2.892
2038	C5	315	16	K	3.034
2039	C5	328	16	K	2.095
2040	C5	334	16	K	2.239
2041	C5	346	16	K	1.836
2042	S1	307	16	K	0.803
2043	S1	312	16	K	0.841
2044	S1	326	16	K	1.59
2045	S1	333	16	K	1.414
2046	S1	335	16	K	0.99
2047	S2	301	16	K	0.913
2048	S2	304	16	K	0.866
2049	S2	308	16	K	0.851
2050	S2	311	16	K	0.932
2051	S2	343	16	K	0.876

Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Kidney Digests (Second Set)
Sample Date 5/27/97 Analysis Date 9/3/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2052	S3	316	16	K	0.726
2053	S3	329	16	K	0.779
2054	S3	331	16	K	0.903
2055	S3	340	16	K	1.079
2056	S3	345	16	K	0.831
2057	S5	305	16	K	1.621
2058	S5	320	16	K	2.042
2059	S5	342	16	K	1.985
2060	S5	344	16	K	1.631
2061	S5	349	16	K	0.972
2062	C3	2322	16	K	1.535
2063	S1	2307	16	K	1.138
2064	CT	338M	16	K	2.985
2065	C1	317H	16	K	3.793

Table II - 8
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Liver Digests
Sample Date 5/27/97 and 5/29/97 Analysis Date 8/11/97 and 8/12/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
1692	CT	302	16	Li	0.888
1693	CT	338	16	Li	0.617
1694	CT	341	16	Li	0.711
1695	C1	306	16	Li	0.444
1696	C1	317	16	Li	0.679
1697	C1	324	16	Li	0.903
1698	C1	327	16	Li	0.42
1699	C1	348	16	Li	1.554
1700	C2	318	16	Li	2.635
1701	C2	319	16	Li	0.605
1702	C2	325	16	Li	2.341
1703	C2	337	16	Li	2.584
1704	C2	339	16	Li	2.094
1705	C3	309	16	Li	3.14
1706	C3	313	16	Li	2.148
1707	C3	322	16	Li	2.35
1708	C3	336	16	Li	2.362
1709	C3	350	16	Li	2.351
1710	C5	303	16	Li	2.832
1711	C5	315	16	Li	11.386
1712	C5	328	16	Li	4.793
1713	C5	334	16	Li	2.057
1714	C5	346	16	Li	3.709
1715	S1	307	16	Li	1.762
1716	S1	312	16	Li	2.806
1717	S1	326	16	Li	1.855
1718	S1	333	16	Li	1.694
1719	S1	335	16	Li	2.117
1720	S2	301	16	Li	2.118
1721	S2	304	16	Li	5.458
1722	S2	308	16	Li	4.174
1723	S2	311	16	Li	2.484
1724	S2	343	16	Li	1.662
1725	S3	316	16	Li	1.679
1726	S3	329	16	Li	7.08
1727	S3	331	16	Li	1.755
1728	S3	340	16	Li	2.022
1729	S3	345	16	Li	1.56
1730	S5	305	16	Li	1.62
1731	S5	320	16	Li	0.858
1732	S5	342	16	Li	1.367
1733	S5	344	16	Li	1.648
1734	S5	349	16	Li	1.673

Table II - 8
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Liver Digests
Sample Date 5/27/97 and 5/29/97 Analysis Date 8/11/97 and 8/12/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
1735	C3	2322	16	Li	1.804
1736	S1	2307	16	Li	2.805
1737	CT	338M	16	Li	3.86
1738	C1	317H	16	Li	5.25
1739	P. Blank				*
1740	P. Blank				*
1741	P. Blank				*

*Because the digestion blanks were so high in nitric acid (too oxidizing) they could not be read by hydride generation without dilution by 50%. This would have resulted in losing the arsenic value by dilution, therefore no arsenic concentration could be measured. If "trace metal" grade nitric acid was used in the digest, there should be no appreciable arsenic contamination.

Table II - 9
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Bile Digests
Sample Date 5/27/97 and 5/29/97 Analysis Date 10/10/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2584	CT	302	16	B	0.748
2585	CT	338	16	B	1.194
2586	CT	341	16	B	1.192
2587	C1	306	16	B	1.431
2588	C1	317	16	B	1.511
2589	C1	324	16	B	2.048
2590	C1	327	16	B	6.914
2591	C1	348	16	B	3.446
2592	C2	318	16	B	2.431
2593	C2	319	16	B	< 0.3
2594	C2	325	16	B	0.652
2595	C2	337	16	B	0.466
2596	C2	339	16	B	0.315
2597	C3	309	16	B	0.336
2598	C3	313	16	B	< 0.3
2599	C3	322	16	B	0.368
2600	C3	336	16	B	0.447
2601	C3	350	16	B	0.123
2602	C5	303	16	B	0.415
2603	C5	315	16	B	0.481
2604	C5	328	16	B	0.54
2605	C5	334	16	B	0.846
2606	C5	346	16	B	0.341
2607	S1	307	16	B	0.272
2608	S1	312	16	B	0.092
2609	S1	326	16	B	1.175
2610	S1	333	16	B	< 0.3
2611	S1	335	16	B	0.21
2612	S2	301	16	B	0.07
2613	S2	304	16	B	0.128
2614	S2	308	16	B	< 0.3
2615	S2	311	16	B	< 0.3
2616	S2	343	16	B	< 0.3

Table II - 9
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Bile Digests
Sample Date 5/27/97 and 5/29/97 Analysis Date 10/10/97

	UMC Sample Description				As
OSU Sample No	Sample ID	Pig No.	Day No.	Tissue	ug/l
2617	S3	316	16	B	0.225
2618	S3	329	16	B	< 0.3
2619	S3	331	16	B	0.288
2620	S3	340	16	B	< 0.3
2621	S3	345	16	B	0.302
2622	S5	305	16	B	0.157
2623	S5	320	16	B	0.389
2624	S5	342	16	B	0.301
2625	S5	344	16	B	0.393
2626	S5	349	16	B	0.354
2627	S5	2349	16	B	0.681
2628	C1	324	16	B	0.307
2629	S1	326L	16	B	0.906
2630	S2	308M	16	B	1.795

Table II - 10
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Lost Arsenic Investigation - Bile and Blood
Collection Date 5/29/97 Analysis Date 10/10/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	Tissue	ug/l
2631	R3-OSU2	322	16	B	3.258
2632	R3-OSU2	314	16	B	0.83
2633	R3-OSU2	2314	16	B	1.872
2634	R3-OSU2	332	16	BL	0.937
2635	R3-OSU2	314	16	BL	0.808
2636	R3-OSU2	347	16	BL	0.958

Table II - 11
Feeding Trial 2: Soils C1, 2, 3, 5 and S1, 2, 3, 5
Lost Arsenic Investigation
Analysis Date 9/4/97

	UMC Sample Description				As
OSU Sample No.	Sample ID	Pig No.	Day No.	ID	ug/l
2073	CT-OSU2	302	15	A	4.266
2074	CT-OSU2	302	15	B	4.938
2075	CT-OSU2	302	15	C	4.286
2076	CT-OSU2	302	15	D	4.13
2077	CT-OSU2	302	15	E	19.24
2078	CT-OSU2	302	15	F	3.008
2079	CT-OSU2	338	15	A	3.135
2080	CT-OSU2	338	15	B	3.287
2081	CT-OSU2	338	15	C	2.887
2082	CT-OSU2	338	15	D	2.925
2083	CT-OSU2	338	15	E	4.908
2084	CT-OSU2	338	15	F	14.254
2085	R3-OSU2	314	15	A	84.951
2086	R3-OSU2	314	15	B	86.395
2087	R3-OSU2	314	15	C	84.333
2088	R3-OSU2	314	15	D	83.394
2089	R3-OSU2	314	15	E	61.9
2090	R3-OSU2	314	15	F	14.402
2091	R3-OSU2	332	15	A	107.67
2092	R3-OSU2	332	15	B	105.19
2093	R3-OSU2	332	15	C	102.39
2094	R3-OSU2	332	15	D	109.31
2095	R3-OSU2	332	15	E	61.538
2096	R3-OSU2	332	15	F	28.502
2097	R3-OSU2	347	15	A	50.67
2098	R3-OSU2	347	15	B	50.04
2099	R3-OSU2	347	15	C	6.052
2100	R3-OSU2	347	15	D	48.34
2101	R3-OSU2	347	15	E	50.978
2102	R3-OSU2	347	15	F	50.844
2103	CT-OSU2	2302	15	D	48.943
2104	CT-OSU2	2302	15	F	4.196
2105	R3-OSU2	2332	15	A	102.23
2106	CT-OSU2	338L	15	A	14.37
2107	R3-OSU2	347M	15	B	76.991

Table II - 12
Assorted Tissues - OSU2B
Sample Date 5/29/97 Analysis Date 7/11/97 and 7/14/97

OSU Sample No.	UMC Sample Description		As
	Pig No.	Tissue	ug/l
1588	314	LI	1.043
1589	2314	LI	0.863
1590	314	LU	1.217
1591	2314	LU	<0.3
1592	314	SP	1.298
1593	2314	SP	1.302
1594	314	KI	4.729
1595	314	TE	<0.3
1596	314	SM	<0.3
1597	314	ST	<0.3
1598	314	HE	<0.3*
1599	314	ID	1.114
1600	314	II	1.032
1601	314	IJ	1.494
1602	314	IC	1.596
1603	314	SC	1.313
1604	314	SK	1.838
1605	2314	SK	2.774
1606	3314	SK	2.492
1607	314	HA	1.616
1608	314	HO	1.496
1609	314	BE	0.751
1610	314	BR	<0.3
1611	312	LI	0.83
1612	312	LU	1.181
1613	2312	LU	1.105
1614	3312	LU	3.502
1615	312	SP	1.262
1616	312	KI	3.604
1617	312	TE	1.002
1618	312	SM	1.166
1619	2312	SM	1.407
1620	312	ST	1.332
1621	312	HE	0.716
1622	312	ID	0.59
1623	312	II	1.042
1624	312	IJ	1.098
1625	312	IC	1.167
1626	312	SC	2.7
1627	312	SK	2.939
1628	312	HA	2.196
1629	312	HO	1.308
1630	312	BE	<0.3
1631	312	BR	<0.3
1632	2312	BR	<0.3

Table II - 12
Assorted Tissues - OSU2B
Sample Date 5/29/97 Analysis Date 7/11/97 and 7/14/97

OSU Sample No.	UMC Sample Description		As
	Pig No.	Tissue	ug/l
1633	347	HZ	2.486
1634	2347	HZ	3.774
1635	3347	HZ	4.021
1636	4347	HZ	3.091
1637	5347	HZ	3.082
1638	6347	HZ	2.874
1639	P. Blank		**
1640	P. Blank		**
1641	P. Blank		**

*Because of the low sample weight on this sample, the digest had to be diluted by 50% to be able to read it by hydride generation. The low concentration result may be therefore due to dilution.

**Because the digestion blanks were so high in nitric acid (much too oxidizing) they could not be read by hydride generation without dilution by 50%. This would have resulted in losing the arsenic value by dilution, therefore no arsenic concentration could be measured. If "trace metal" grade nitric acid was used in the digest, there should be no appreciable arsenic contamination.

Table II - 13
Soil Dosing - Feeding Trial 1

Group	Treatment	As intake ug/kg/day	Pig #
1	Na2AsO4.7H2O	10	222
			227
			235
			249
			251
2	Na2AsO4.7H2O	30	216
			218
			225
			236
			240
3	Na2AsO4.7H2O	90	205
			208
			215
			224
4	Site 1 Soil Calcine C-4	40	201
			206
			217
			223
			238
5	Site 1 Soil C-4	80	214
			233
			241
			246
			248
6	Site 1 Soil C-4	160	202
			203
			229
			231
			250
7	Site 2 Soil Slag S-4	40	204
			228
			237
			239
			253
8	Site 2 Soil S-4	80	207
			212
			213
			220
			244
9	Site 2 Soil S-4	160	211
			219
			232
			234
			242
10	Control	0	221
			230
			243

Appendix III

Chemical Speciation Data

Table III - 1
Water Soluble Extracts
1 g soil:20 ml, shake 1 hour, room temperature

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected As, mg/L	As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, % Ind.	Bioavable As, % Ave
2206	C1	< 250 u	1	0.0647	0.0664	1.33	1.13	0.183	11294	0.012	0.010
2207	C1	< 250 u	2	0.0470	0.0482	0.96				0.009	
2208	C1	< 250 u	3	0.0540	0.0554	1.11				0.010	
2209	C2	< 250 u	1	0.0591	0.0606	1.21	1.25	0.097	17456	0.007	0.007
2210	C2	< 250 u	2	0.0660	0.0677	1.35				0.008	
2211	C2	< 250 u	3	0.0570	0.0585	1.17				0.007	
2212	C3	< 250 u	1	0.0290	0.0298	0.60	0.60	0.041	13472	0.004	0.004
2213	C3	< 250 u	2	0.0270	0.0277	0.55				0.004	
2214	C3	< 250 u	3	0.0310	0.0318	0.64				0.005	
1748	C4	< 250 u	1	0.0384	0.0394	0.79	0.78	0.050	11525	0.007	0.007
1749	C4	< 250 u	2	0.0404	0.0414	0.83				0.007	
1750	C4	< 250 u	3	0.0355	0.0364	0.73				0.006	
2215	C5	< 250 u	1	0.0067	0.0069	0.14	0.14	0.000	6245	0.002	0.002
2216	C5	< 250 u	2	0.0067	0.0069	0.14				0.002	
2217	C5	< 250 u	3	0.0067	0.0069	0.14				0.002	
2218	C4	< 2mm	1	0.0340	0.0349	0.70	0.73	0.031	10918	0.006	0.007
2219	C4	< 2mm	2	0.0350	0.0359	0.72				0.007	
2220	C4	< 2mm	3	0.0370	0.0380	0.76				0.007	
2221	S1	< 250 u	1	0.0360	0.0369	0.74	0.74	0.308	405	0.182	0.182
2222	S1	< 250 u	2	0.0510	0.0523	1.05				0.258	
2223	S1	< 250 u	3	0.0210	0.0215	0.43				0.106	
2224	S2	< 250 u	1	0.1100	0.1129	2.26	2.54	0.256	450	0.502	0.565
2225	S2	< 250 u	2	0.1280	0.1313	2.63				0.584	
2226	S2	< 250 u	3	0.1340	0.1375	2.75				0.611	
2227	S3	< 250 u	1	0.0880	0.0903	1.81	1.76	0.188	1180	0.153	0.150
2228	S3	< 250 u	2	0.0760	0.0780	1.56				0.132	
2229	S3	< 250 u	3	0.0940	0.0964	1.93				0.163	

Table III - 1
Water Soluble Extracts
1 g soil:20 ml, shake 1 hour, room temperature

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected As, mg/L	As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, % Ind.	Bioavable As, % Ave
1766	S4	< 250 u	1	~~0.1181~~	~~0.1212~~	~~2.42~~	3.12	1.325	5022	~~0.048~~	0.062
1767	S4	< 250 u	2	0.2392	0.2454	4.91				0.098	
1768	S4	< 250 u	3	0.2174	0.2231	4.46				0.089	
2230	S5	< 250 u	1	0.1810	0.1857	3.71	3.54	0.211	4650	0.080	0.076
2231	S3	< 250 u	2	0.1750	0.1796	3.59				0.077	
2232	S5	< 250 u	3	0.1610	0.1652	3.30				0.071	
2233	S4	< 2mm	1	0.1800	0.1847	3.69	3.77	0.113	3158	0.117	0.119
2234	S4	< 2mm	2	0.1810	0.1857	3.71				0.118	
2235	S4	< 2mm	3	0.1900	0.1949	3.90				0.123	
2236	E1	< 250 u	1	0.0040	0.0041	0.08	0.08	0.012	331	0.025	0.023
2237	E1	< 250 u	2	0.0030	0.0031	0.06				0.019	
2238	E1	< 250 u	3	0.0040	0.0041	0.08				0.025	
2239	E2	< 250 u	1	0.0220	0.0226	0.45	1.66	2.115	233	0.194	0.713
2240	E2	< 250 u	2	0.0210	0.0215	0.43				0.185	
2241	E2	< 250 u	3	0.2000	0.2052	4.10				1.761	
2242	E3	< 250 u	1	0.0080	0.0082	0.16	0.16	0.000	800	0.021	0.021
2243	E3	< 250 u	2	0.0080	0.0082	0.16				0.021	
2244	E3	< 250 u	3	0.0080	0.0082	0.16				0.021	
2245	E4	< 250 u	1	0.0280	0.0287	0.57	0.49	0.198	1463	0.039	0.034
2246	E4	< 250 u	2	0.0310	0.0318	0.64				0.043	
2247	E4	< 250 u	3	0.0130	0.0133	0.27				0.018	
2248	E5	< 250 u	1	0.0680	0.0698	1.40	1.30	0.085	401	0.348	0.324
2249	E5	< 250 u	2	0.0600	0.0616	1.23				0.307	
2250	E5	< 250 u	3	0.0620	0.0636	1.27				0.317	
2251	BG	< 2mm	1	0.0160	0.0164	0.33	0.31	0.012			
2252	BG	< 2mm	2	0.0150	0.0154	0.31					
2253	BG	< 2mm	3	0.0150	0.0154	0.31					

Table III - 2
Sodium Acetate Extracts
1g:20 ml, shake 1 hr, rt

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected* As, mg/L	Individual As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, mg/kg Indiv.	Ave.
1802	S4	< 250 u	1	6.242	6.4043	128.086	127.402	0.687	5022	2.550	2.54
1803	S4	< 250 u	2	6.175	6.3356	126.711				2.523	
1804	S4	< 250 u	3	6.209	6.3704	127.409				2.537	
2129	S5	< 250 u	1	3.886	3.9870	79.741	82.976	2.941	4650	1.715	1.78
2130	S3	< 250 u	2	4.166	4.2743	85.486				1.838	
2131	S5	< 250 u	3	4.079	4.1851	83.701				1.800	
2135	S4	< 2mm	1	5.188	5.3229	106.458	107.518	0.972	3158	3.371	3.40
2136	S4	< 2mm	2	5.25	5.3865	107.730				3.411	
2137	S4	< 2mm	3	5.281	5.4183	108.366				3.431	
2141	E1	< 250 u	1	1.641	1.6837	33.673	32.579	1.041	331	10.173	9.84
2142	E1	< 250 u	2	1.582	1.6231	32.463				9.807	
2143	E1	< 250 u	3	1.54	1.5800	31.601				9.547	
2144	E2	< 250 u	1	2.044	2.0971	41.943	40.445	2.612	233	18.001	17.36
2145	E2	< 250 u	2	2.045	2.0982	41.963				18.010	
2146	E2	< 250 u	3	1.824	1.8714	37.428				16.064	
2147	E3	< 250 u	1	2.688	2.7579	55.158	56.232	1.685	800	6.895	7.03
2148	E3	< 250 u	2	2.835	2.9087	58.174				7.272	
2149	E3	< 250 u	3	2.698	2.7681	55.363				6.920	
2150	E4	< 250 u	1	9.302	9.5439	190.877	195.268	4.072	1463	13.047	13.35
2151	E4	< 250 u	2	9.552	9.8004	196.007				13.398	
2152	E4	< 250 u	3	9.694	9.9460	198.921				13.597	
2153	E5	< 250 u	1	2.303	2.3629	47.258	47.593	1.116	401	11.785	11.87
2154	E5	< 250 u	2	2.38	2.4419	48.838				12.179	
2155	E5	< 250 u	3	2.275	2.3342	46.683				11.642	
2138	BG	< 2mm	1	1.618	1.6601	33.201	32.866	0.431			
2139	BG	< 2mm	2	1.609	1.6508	33.017					
2140	BG	< 2mm	3	1.578	1.6190	32.381					

Table III - 2
Sodium Acetate Extracts
1g:20 ml, shake 1 hr, rt

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected* As, mg/L	Individual As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, mg/kg Indiv.	Ave.
2108	C1	< 250 u	1	2.088	2.1423	42.846	41.649	1.041	11294	0.379	0.37
2109	C1	< 250 u	2	2.005	2.0571	41.143				0.364	
2110	C1	< 250 u	3	1.996	2.0479	40.958				0.363	
2111	C2	< 250 u	1	2.131	2.1864	43.728	44.610	2.046	17456	0.251	0.26
2112	C2	< 250 u	2	2.103	2.1577	43.154				0.247	
2113	C2	< 250 u	3	2.288	2.3475	46.950				0.269	
2114	C3	< 250 u	1	2.278	2.3372	46.745	43.721	2.636	13472	0.347	0.32
2115	C3	< 250 u	2	2.072	2.1259	42.517				0.316	
2116	C3	< 250 u	3	2.042	2.0951	41.902				0.311	
1784	C4	< 250 u	1	2.447	2.5106	50.212	52.921	2.569	11525	0.436	0.46
1785	C4	< 250 u	2	2.696	2.7661	55.322				0.480	
1786	C4	< 250 u	3	2.594	2.6614	53.229				0.462	
2117	C5	< 250 u	1	1.727	1.7719	35.438	34.966	0.871	6245	0.567	0.56
2118	C5	< 250 u	2	1.73	1.7750	35.500				0.568	
2119	C5	< 250 u	3	1.655	1.6980	33.961				0.544	
2132	C4	< 2mm	1	2.033	2.0859	41.717	41.259	0.442	10918	0.382	0.38
2133	C4	< 2mm	2	1.99	2.0417	40.835				0.374	
2134	C4	< 2mm	3	2.009	2.0612	41.225				0.378	
2120	S1	< 250 u	1	4.395	4.5093	90.185	88.770	1.960	405	22.268	21.92
2121	S1	< 250 u	2	4.217	4.3266	86.533				21.366	
2122	S1	< 250 u	3	4.366	4.4795	89.590				22.121	
2123	S2	< 250 u	1	2.825	2.8985	57.969	58.112	1.045	450	12.882	12.91
2124	S2	< 250 u	2	2.886	2.9610	59.221				13.160	
2125	S2	< 250 u	3	2.793	2.8572	57.145				12.699	
2126	S3	< 250 u	1	3.804	3.9029	78.058	81.150	7.607	1180	6.615	6.88
2127	S3	< 250 u	2	3.683	3.7788	75.575				6.405	
2128	S3	< 250 u	3	4.377	4.4908	89.816				7.612	

Table III - 3
Yamoto Phosphate
1 g:20 ml, shake 1 hr, room temperature

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected* As, mg/L	Individual As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, %	
										Indiv.	Ave.
2157	C1	< 250 u	1	5.562	5.7066	114.132	117.484	2.963	11294	1.011	1.040
2158	C1	< 250 u	2	5.778	5.9282	118.565				1.050	
2159	C1	< 250 u	3	5.836	5.9877	119.755				1.060	
2160	C2	< 250 u	1	5.833	5.9847	119.693	122.183	4.745	11294	1.060	1.082
2161	C2	< 250 u	2	5.809	5.9600	119.201				1.055	
2162	C2	< 250 u	3	6.221	6.3827	127.655				1.130	
2163	C3	< 250 u	1	7.262	7.4508	149.016	151.403	2.213	11294	1.319	1.341
2164	C3	< 250 u	2	7.398	7.5903	151.807				1.344	
2165	C3	< 250 u	3	7.475	7.6694	153.387				1.358	
1856	C4	< 250 u	1	10.806	11.0870	221.739	219.297	3.575	11294	1.963	1.942
1857	C4	< 250 u	2	10.768	11.0480	220.959				1.956	
1858	C4	< 250 u	3	10.487	10.7597	215.193				1.905	
2166	C5	< 250 u	1	10.2	10.4652	209.304	224.489	16.935	11294	1.853	1.988
2167	C5	< 250 u	2	10.79	11.0705	221.411				1.960	
2168	C5	< 250 u	3	11.83	12.1376	242.752				2.149	
2181	C4	< 2mm	1	12.266	12.5849	251.698	254.838	2.719	11294	2.229	2.256
2182	C4	< 2mm	2	12.494	12.8188	256.377				2.270	
2183	C4	< 2mm	3	12.497	12.8219	256.438				2.271	
2169	S1	< 250 u	1	3.576	3.6690	73.380	74.419	1.592	11294	0.650	0.659
2170	S1	< 250 u	2	3.716	3.8126	76.252				0.675	
2171	S1	< 250 u	3	3.588	3.6813	73.626				0.652	
2172	S2	< 250 u	1	4.38	4.4939	89.878	81.644	11.644	11294	0.796	0.723
2173	S2	< 250 u	2	lost	---	---				---	
2174	S2	< 250 u	3	4.38	3.6705	73.410				0.650	
2175	S3	< 250 u	1	4.302	4.4939	89.878	88.099	2.296	11294	0.796	0.780
2176	S3	< 250 u	2	4.167	4.2753	85.507				0.757	
2177	S3	< 250 u	3	4.333	4.4457	88.913				0.787	

Table III - 3
Yamoto Phosphate
1 g:20 ml, shake 1 hr, room temperature

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected* As, mg/L	Individual As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavable As, % Indiv.	Bioavable As, % Ave.
1874	S4	< 250 u	1	6.397	4.4139	88.277	120.637	28.070	11294	0.782	1.068
1875	S4	< 250 u	2	6.59	6.7613	135.227				1.197	
1876	S4	< 250 u	3	6.745	6.9204	138.407				1.225	
2178	S5	< 250 u	1	5.363	5.5024	110.049	107.196	3.971	11294	0.974	0.949
2179	S3	< 250 u	2	5.003	5.1331	102.662				0.909	
2180	S5	< 250 u	3	5.306	5.4440	108.879				0.964	
2184	S4	< 2mm	1	4.676	4.7976	95.952	97.641	1.485	11294	0.850	0.865
2185	S4	< 2mm	2	4.787	4.9115	98.229				0.870	
2186	S4	< 2mm	3	4.812	4.9371	98.742				0.874	
2190	E1	< 250 u	1	3.392	3.4802	69.604	70.048	0.735	11294	0.616	0.620
2191	E1	< 250 u	2	3.394	3.4822	69.645				0.617	
2192	E1	< 250 u	3	3.455	3.5448	70.897				0.628	
2193	E2	< 250 u	1	3.895	3.9963	79.925	79.905	0.749	11294	0.708	0.707
2194	E2	< 250 u	2	3.857	3.9573	79.146				0.701	
2195	E2	< 250 u	3	3.93	4.0322	80.644				0.714	
2196	E3	< 250 u	1	5.65	5.7969	115.938	120.904	5.846	11294	1.027	1.071
2197	E3	< 250 u	2	5.82	5.9713	119.426				1.057	
2198	E3	< 250 u	3	6.206	6.3674	127.347				1.128	
2199	E4	< 250 u	1	3.895	3.9963	79.925	78.776	1.714	11294	0.708	0.698
2200	E4	< 250 u	2	3.743	3.8403	76.806				0.680	
2201	E4	< 250 u	3	3.879	3.9799	79.597				0.705	
2202	E5	< 250 u	1	4.695	4.8171	96.341	96.916	2.012	11294	0.853	0.858
2203	E5	< 250 u	2	4.832	4.9576	99.153				0.878	
2204	E5	< 250 u	3	4.642	4.7627	95.254				0.843	
2187	BG	< 2mm	1	2.896	2.9713	59.426	61.690	2.058			
2188	BG	< 2mm	2	3.092	3.1724	63.448					
2189	BG	< 2mm	3	3.031	3.1098	62.196					

2205 blank < 0.133

Table III - 4
Hydroxylamine HCl
1 g:250 ml, shake 2 hr, 70 C

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected As, mg/L	As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavailable As, % Indiv.	Bioavailable As, % Ave.
2254	C1	< 250 u	1	6.838	7.0158	1753.947	1717.011	398.348	11294	15.53	15.20
2255	C1	< 250 u	2	8.17	8.3824	2095.605				18.56	
2256	C1	< 250 u	3	5.074	5.2059	1301.481				11.52	
2257	C2	< 250 u	1	10.412	10.6827	2670.678	2929.572	376.462	17456	15.30	16.78
2258	C2	< 250 u	2	13.105	13.4457	3361.433				19.26	
2259	C2	< 250 u	3	10.747	11.0264	2756.606				15.79	
2260	C3	< 250 u	1	11.351	11.6461	2911.532	3018.407	426.715	13472	21.61	22.41
2261	C3	< 250 u	2	13.6	13.9536	3488.400				25.89	
2262	C3	< 250 u	3	10.352	10.6212	2655.288				19.71	
2263	C4	< 250 u	1	19.037	19.5320	4882.991	4930.443	191.326	11525	42.37	42.78
2264	C4	< 250 u	2	20.043	20.5641	5141.030				44.61	
2265	C4	< 250 u	3	18.586	19.0692	4767.309				41.36	
2266	C5	< 250 u	1	11.833	12.1407	3035.165	3084.926	211.434	6245	48.60	49.40
2267	C5	< 250 u	2	11.317	11.6112	2902.811				46.48	
2268	C5	< 250 u	3	12.931	13.2672	3316.802				53.11	
2284	C4	< 2 mm	1	16.121	16.5401	4135.037	4688.478	806.032	4650	88.93	100.83
2285	C4	< 2 mm	2	16.831	17.2686	4317.152				92.84	
2286	C4	< 2 mm	3	21.884	22.4530	5613.246				120.71	
2269	S1	< 250 u	1	1.332	1.3666	341.658	383.895	43.530	10918	3.13	3.52
2270	S1	< 250 u	2	1.671	1.7144	428.612				3.93	
2271	S1	< 250 u	3	1.487	1.5257	381.416				3.49	
2272	S2	< 250 u	1	1.64	1.6826	420.660	413.363	30.085	405	103.87	102.06
2273	S2	< 250 u	2	1.712	1.7565	439.128				108.43	
2274	S2	< 250 u	3	3.348	1.5212	380.300				93.90	
2275	S3	< 250 u	1	3.348	1.6826	420.660	700.844	242.992	450	93.48	155.74
2276	S3	< 250 u	2	3.329	3.4156	853.889				189.75	
2277	S3	< 250 u	3	3.228	3.3119	827.982				184.00	

Table III - 4
Hydroxylamine HCl
1 g:250 ml, shake 2 hr, 70 C

Sample No.	Soil	Particle Size	Rep No.	As mg/L	Corrected As, mg/L	As, mg/kg	Average As, mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavailable As, % Indiv.	Bioavailable As, % Ave.
2278	S4	< 250 u	1	15.422	15.8230	3955.743	3650.936	269.385	1180	335.23	309.40
2279	S4	< 250 u	2	13.849	14.2091	3552.269				301.04	
2280	S4	< 250 u	3	13.43	13.7792	3444.795				291.93	
2281	S5	< 250 u	1	11.641	11.9437	2985.917	2940.089	217.571	5022	59.46	58.54
2282	S5	< 250 u	2	10.539	10.8130	2703.254				53.83	
2283	S5	< 250 u	3	12.207	12.5244	3131.096				62.35	
2287	S4	< 2 mm	1	9.747	10.0004	2500.106	2665.805	147.558	3158	79.17	84.41
2288	S4	< 2 mm	2	10.85	11.1321	2783.025				88.13	
2289	S4	< 2 mm	3	10.582	10.8571	2714.283				85.95	
2293	E1	< 250 u	1	0.779	0.7993	199.814	186.048	13.135	331	85.76	79.85
2294	E1	< 250 u	2	0.677	0.6946	173.651				74.53	
2295	E1	< 250 u	3	0.72	0.7387	184.680				79.26	
2296	E2	< 250 u	1	0.685	0.7028	175.703	207.338	41.399	233	21.96	25.92
2297	E2	< 250 u	2	0.749	0.7685	192.119				24.01	
2298	E2	< 250 u	3	0.991	1.0168	254.192				31.77	
2299	E3	< 250 u	1	2.888	2.9631	740.772	725.468	16.081	800	50.63	49.59
2300	E3	< 250 u	2	2.763	2.8348	708.710				48.44	
2301	E3	< 250 u	3	2.834	2.9077	726.921				49.69	
2302	E4	< 250 u	1	4.708	4.8304	1207.602	1213.587	9.278	1463	301.15	302.64
2303	E4	< 250 u	2	4.713	4.8355	1208.885				301.47	
2304	E4	< 250 u	3	4.773	4.8971	1224.275				305.31	
2305	E5	< 250 u	1	1.241	1.2733	318.317	308.399	10.005	401	79.38	76.91
2306	E5	< 250 u	2	1.163	1.1932	298.310				74.39	
2307	E5	< 250 u	3	1.203	1.2343	308.570				76.95	
2290	BG	< 2 mm	1	0.124	0.1272	31.806	33.174	16.970			
2291*	BG	< 2 mm	2	0.066	0.0677	16.929					
2292	BG	< 2 mm	3	0.198	0.2031	50.787					
2308	blank			< 0.133							

*As concentration represents 1/2 detection limit as this was too low to read.

Table III - 5
Ammonium Oxalate/Oxalic Acid/Ascorbic Acid Extracts
1 g:100 ml, 15 min @ 97 C

Sample No.	Soil	Particle Size	Rep No.	As		As, Ave	Standard	Method 3050	Bioavailable As, %	
				mg/L	mg/kg	mg/kg	Deviation	As, mg/kg	Indiv.	Ave.
2479	C1	< 250 u	1	75.606	7560.60	7454.07	177.30	11294	66.94	66.00
2480	C1	< 250 u	2	72.494	7249.40				64.19	
2481	C1	< 250 u	3	75.522	7552.20				66.87	
2482	C2	< 250 u	1	115.27	11527.30	12616.03	992.28	17456	66.04	72.27
2483	C2	< 250 u	2	134.7	13469.60				77.16	
2484	C2	< 250 u	3	128.51	12851.20				73.62	
2485	C3	< 250 u	1	85.898	8589.80	9046.93	462.13	13472	63.76	67.15
2486	C3	< 250 u	2	90.371	9037.10				67.08	
2487	C3	< 250 u	3	95.139	9513.90				70.62	
2488	C4	< 250 u	1	80.06	8006.00	8129.97	728.01	11525	69.47	70.54
2489	C4	< 250 u	2	89.12	8912.00				77.33	
2490	C4	< 250 u	3	74.719	7471.90				64.83	
2491	C5	< 250 u	1	48.248	4824.80	4951.30	216.94	6245	77.26	79.28
2492	C5	< 250 u	2	48.273	4827.30				77.30	
2493	C5	< 250 u	3	52.018	5201.80				83.30	
2509	C4	< 2mm	1	52.536	5253.60	5402.87	371.09	10918	48.12	49.49
2510	C4	< 2mm	2	52.151	5215.10				47.77	
2511	C4	< 2mm	3	57.399	5739.90				52.57	
2494	S1	< 250 u	1	4.29	429.00	408.63	17.65	405	105.93	100.90
2495	S1	< 250 u	2	3.991	399.10				98.54	
2496	S1	< 250 u	3	3.978	397.80				98.22	
2497	S2	< 250 u	1	5.324	532.40	545.93	12.86	450	118.31	121.32
2498	S2	< 250 u	2	5.58	558.00				124.00	
2499	S2	< 250 u	3	5.474	547.40				121.64	
2500	S3	< 250 u	1	12.855	1285.50	1213.97	73.36	1180	108.94	102.88
2501	S3	< 250 u	2	11.389	1138.90				96.52	
2502	S3	< 250 u	3	12.175	1217.50				103.18	

Table III - 5
Ammonium Oxalate/Oxalic Acid/Ascorbic Acid Extracts
1 g:100 ml, 15 min @ 97 C

Sample No.	Soil	Particle Size	Rep No.	As mg/L	As mg/kg	As, Ave mg/kg	Standard Deviation	Method 3050 As, mg/kg	Bioavailable As, % Indiv.	Bioavailable As, % Ave.
2503	S4	< 250 u	1	39.662	3966.20	4030.07	117.53	5022	78.98	80.25
2504	S4	< 250 u	2	41.657	4165.70				82.95	
2505	S4	< 250 u	3	39.583	3958.30				78.82	
2506	S5	< 250 u	1	33.028	3302.80	3330.57	25.99	4650	71.03	71.63
2507	S5	< 250 u	2	33.346	3334.60				71.71	
2508	S5	< 250 u	3	33.543	3354.30				72.14	
2512	S4	< 2mm	1	19.426	1942.60	2134.73	44.26	3158	61.51	67.60
2513	S4	< 2mm	2	21.995	2199.50				69.65	
2514	S4	< 2mm	3	22.621	2262.10				71.63	
2518	E1	< 250 u	1	4.634	463.40	461.47	15.89	331	140.00	139.42
2519	E1	< 250 u	2	4.763	476.30				143.90	
2520	E1	< 250 u	3	4.447	444.70				134.35	
2521	E2	< 250 u	1	4.551	455.10	454.33	5.29	233	195.32	194.99
2522	E2	< 250 u	2	4.487	448.70				192.58	
2523	E2	< 250 u	3	4.592	459.20				197.08	
2524	E3	< 250 u	1	5.941	594.10	591.37	10.76	800	74.26	73.92
2525	E3	< 250 u	2	6.005	600.50				75.06	
2526	E3	< 250 u	3	5.795	579.50				72.44	
2527	E4	< 250 u	1	4.111	411.10	437.93	25.28	1463	28.10	29.93
2528	E4	< 250 u	2	4.613	461.30				31.53	
2529	E4	< 250 u	3	4.414	441.40				30.17	
2530	E5	< 250 u	1	5.678	567.80	551.73	18.91	401	141.60	137.59
2531	E5	< 250 u	2	5.565	556.50				138.78	
2532	E5	< 250 u	3	5.309	530.90				132.39	
2515	BG	< 2mm	1	2.914	291.40	287.77	1.34			
2516	BG	< 2mm	2	2.869	286.90					
2517	BG	< 2mm	3	2.85	285.00					
2533	lank			0.25						

Table III - 6
Sodium Hydroxide/Sodium Chloride Extracts
0.8 g:40 ml, shake 17 hr @ RT, dilute to 50 ml

Sample No.	Soil	Particle Size	Rep No.	As			Standard Deviation	3505 As mg/kg	Bioavailable As, %	
				mg/L	mg/kg	Ave, mg/kg			Indiv.	Ave.
2771	C1	< 250 u	1	17.14	1071.25	1017.58	64.997	11294	9.485	9.01
2772	C1	< 250 u	2	16.579	1036.19				9.175	
2773	C1	< 250 u	3	15.125	945.31				8.370	
2774	C2	< 250 u	1	29.863	1866.44	1818.54	51.541	17456	10.692	10.42
2775	C2	< 250 u	2	28.224	1764.00				10.105	
2776	C2	< 250 u	3	29.203	1825.19				10.456	
2777	C3	< 250 u	1	24.7	1543.75	1468.27	65.388	13472	11.459	10.90
2778	C3	< 250 u	2	22.862	1428.88				10.606	
2779	C3	< 250 u	3	22.915	1432.19				10.631	
2780	C4	< 250 u	1	8.639	539.94	528.21	12.645	11525	4.685	4.58
2781	C4	< 250 u	2	8.237	514.81				4.467	
2782	C4	< 250 u	3	8.478	529.88				4.598	
2783	C5	< 250 u	1	5.148	321.75	317.73	3.632	6245	5.152	5.09
2784	C5	< 250 u	2	5.035	314.69				5.039	
2785	C5	< 250 u	3	5.068	316.75				5.072	
2801	C4	< 2 mm	1	0	0.00	187.63		10918	0.000	0.57
2802	C4	< 2 mm	2	3.002	187.63				1.718	
2803	C4	< 2 mm	3	0	0.00				0.000	
2786	S1	< 250 u	1	3.092	193.25	196.69	3.241	405	47.716	48.56
2787	S1	< 250 u	2	3.154	197.13				48.673	
2788	S1	< 250 u	3	3.195	199.69				49.306	
2789	S2	< 250 u	1	3.185	199.06	200.90	1.983	450	44.236	44.64
2790	S2	< 250 u	2	3.21	200.63				44.583	
2791	S2	< 250 u	3	3.248	203.00				45.111	
2792	S3	< 250 u	1	3.765	235.31	236.17	3.853	1180	19.942	20.01
2793	S3	< 250 u	2	3.725	232.81				19.730	
2794	S3	< 250 u	3	3.846	240.38				20.371	

Table III - 6
Sodium Hydroxide/Sodium Chloride Extracts
0.8 g:40 ml, shake 17 hr @ RT, dilute to 50 ml

Sample No.	Soil	Particle Size	Rep No.	As			Standard Deviation	3505 As mg/kg	Bioavailable As, %	
				mg/L	mg/kg	Ave, mg/kg			Indiv.	Ave.
2795	S4	< 250 u	1	5.15	321.88	328.10	9.264	5022	6.409	6.53
2796	S4	< 250 u	2	5.42	338.75				6.745	
2797	S4	< 250 u	3	5.179	323.69				6.445	
2798	S5	< 250 u	1	4.768	298.00	300.63	2.565	4650	6.409	6.47
2799	S5	< 250 u	2	4.85	303.13				6.519	
2800	S5	< 250 u	3	4.812	300.75				6.468	
2804	S4	< 2 mm	1	3.052	190.75	190.88	110.202	3158	6.040	4.03
2805	S4	< 2 mm	2	0	0.00				0.000	
2806	S4	< 2 mm	3	3.056	191.00				6.048	
2807	E1	< 250 u	1	3.446	215.38	212.90	2.147	331	65.068	64.32
2808	E1	< 250 u	2	3.387	211.69				63.954	
2809	E1	< 250 u	3	3.386	211.63				63.935	
2810	E2	< 250 u	1	3.314	207.13	210.81	3.356	233	88.895	90.48
2811	E2	< 250 u	2	3.386	211.63				90.826	
2812	E2	< 250 u	3	3.419	213.69				91.711	
2813	E3	< 250 u	1	3.082	192.63	195.19	2.967	800	24.078	24.40
2814	E3	< 250 u	2	3.175	198.44				24.805	
2815	E3	< 250 u	3	3.112	194.50				24.313	
2816	E4	< 250 u	1	6.917	432.31	462.54	27.311	1463	29.550	31.62
2817	E4	< 250 u	2	7.767	485.44				33.181	
2818	E4	< 250 u	3	7.518	469.88				32.117	
2819	E5	< 250 u	1	4.376	273.50	274.58	1.823	401	68.204	68.47
2820	E5	< 250 u	2	4.427	276.69				68.999	
2821	E5	< 250 u	3	4.377	273.56				68.220	

Table III - 7

Raw data for 3050 extracts of 250 micron fraction. Concentrations are in ppm.

Sample	Sample ID	As			Pb			Cu			Ni		
No.	ID	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev
222	C1-1	112.94	11294	11366	120.98	12098	11976	4.11	411	403	0.38	38	34
223	C1-2	115.31	11531	143	119.94	11994	132	4.02	402	7	0.25	25	8
224	C1-3	112.73	11273		118.36	11836		3.97	397		0.39	39	
225	C2-1	174.56	17456	17279	149.74	14974	14847	3.53	353	351	0.55	55	36
226	C2-2	175.37	17537	379	151.89	15189	420	3.6	360	11	0.25	25	16
227	C2-3	168.43	16843		143.79	14379		3.39	339		0.29	29	
228	C3-1	134.72	13472	13518	114.72	11472	11640	3.95	395	402	0.2	20	23
229	C3-2	136.62	13662	127	118.13	11813	171	4.11	411	8	0.3	30	6
230	C3-3	134.2	13420		116.35	11635		4.01	401		0.19	19	
231	C4-1	115.25	11525	11648	91.52	9152	9204	4.93	493	495	0.6	60	31
232	C4-2	115.9	11590	161	91.85	9185	63	4.9	490	6	0.17	17	25
233	C4-3	118.3	11830		92.74	9274		5.02	502		0.16	16	
234	C5-1	62.45	6245	6607	52.55	5255	5528	6.77	677	713	0.27	27	25
235	C5-2	66.72	6672	334	56.16	5616	241	7.19	719	34	0.27	27	3
236	C5-3	69.04	6904		57.13	5713		7.44	744		0.21	21	
237	Blank	0	0		0.15			0.03			0.15		
238	S1-1	4.05	405	466	62.77	6277	6738	13.24	1324	1422	0.53	53	37
239	S1-2	4.9	490	53	69.1	6910	403	14.71	1471	85	0.29	29	14
240	S1-3	5.02	502		70.26	7026		14.7	1470		0.29	29	
241	S2-1	4.5	450	422	40.41	4041	3845	12.04	1204	1151	0.23	23	50
242	S2-2	3.83	383	35	36.77	3677	184	10.92	1092	56	0.87	87	33
243	S2-3	4.32	432		38.17	3817		11.57	1157		0.39	39	
244	S3-1	11.8	1180	1213	52.6	5260	5379	23.71	2371	2445	0.35	35	41
245	S3-2	12.17	1217	31	54	5400	110	24.68	2468	66	0.64	64	20
246	S3-3	12.41	1241		54.78	5478		24.96	2496		0.25	25	

Table III - 7

Raw data for 3050 extracts of 250 micron fraction. Concentrations are in ppm.

Sample	Sample ID	As			Pb			Cu			Ni		
No.	ID	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev
247	S4-1	50.22	5022	5068	210.73	21073	20412	55.22	5522	5359	0.31	31	40
248	S4-2	50.96	5096	40	201.37	20137	575	53.54	5354	160	0.47	47	8
249	S4-3	50.87	5087		200.26	20026		52.02	5202		0.41	41	
250	S5-1	46.5	4650	4470	193.92	19392	18930	52.97	5297	5307	0.33	33	44
251	S5-2	43.5	4350	159	187.16	18716	400	53.52	5352	41	0.53	53	10
252	S5-3	44.11	4411		186.83	18683		52.71	5271		0.46	46	
253	Blank	0			0.2			0.07			0.12		

Table III - 7

Sample No.	Sample ID ID	Ti			Zn		
		Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev
222	C1-1	1.23	123	116	19.44	1944	1942
223	C1-2	1.09	109	7	19.36	1936	6
224	C1-3	1.15	115		19.47	1947	
225	C2-1	1.07	107	108	20.37	2037	2065
226	C2-2	1.07	107	2	21.3	2130	56
227	C2-3	1.11	111		20.28	2028	
228	C3-1	1.39	139	140	18.89	1889	1893
229	C3-2	1.38	138	2	18.89	1889	7
230	C3-3	1.42	142		19.01	1901	
231	C4-1	1.8	180	171	18.11	1811	1841
232	C4-2	1.61	161	10	18.52	1852	26
233	C4-3	1.73	173		18.6	1860	
234	C5-1	2.41	241	265	48.03	4803	5051
235	C5-2	2.85	285	22	50.74	5074	238
236	C5-3	2.68	268		52.77	5277	
237	Blank	0.01			0		
238	S1-1	6.79	679	712	249.05	24905	25872
239	S1-2	7.01	701	40	261.87	26187	854
240	S1-3	7.56	756		265.23	26523	
241	S2-1	5.85	585	574	145.46	14546	13910
242	S2-2	5.63	563	11	136.51	13651	554
243	S2-3	5.74	574		135.32	13532	
244	S3-1	4.89	489	532	25.78	2578	2640
245	S3-2	5.45	545	39	26.37	2637	63
246	S3-3	5.63	563		27.04	2704	

Table III - 7

Sample No.	Sample ID ID	Ti			Zn		
		Solution	Soil	Ave/StDev	Solution	Soil	Ave/StDev
247	S4-1	3.98	398	404	50.44	5044	5166
248	S4-2	4.11	411	7	52.27	5227	106
249	S4-3	4.03	403		52.27	5227	
250	S5-1	3.67	367	364	45.84	4584	4511
251	S5-2	3.59	359	4	44.49	4449	68
252	S5-3	3.66	366		45	4500	
253	Blank	0.01			0		

Table III - 8

Raw data for 3050 extracts of 2 mm fraction. Concentrations are mg/l in solution and mg/kg soil.

Sample No.	Sample ID	As		Cr		Cu		Ni	
		mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg
1383	C1-1	11309.6	11301.67	30.3	29.43	385.4	385.13	39.3	39.10
1384	C1-2	11251.6		27.6		373		36.6	
1385	C1-3	11343.8		30.4		397		41.4	
1386	C2-1	16314.6	15995.53	22.1	21.60	327.6	318.87	35.3	35.93
1387	C2-2	16005.3		21.6		318.7		36.7	
1388	C2-3	15666.7		21.1		310.3		35.8	
1389	C3-1	14516.3	13418.53	27.4	25.83	392.8	384.13	31.8	31.40
1390	C3-2	12695		24.7		371.3		30.9	
1391	C3-3	13044.3		25.4		388.3		31.5	
1392	C4-1	10958.4	10918.27	25.8	25.40	541.4	524.27	30.5	32.17
1393	C4-2	10748.1		25.2		530.7		31.1	
1394	C4-3	11048.3		25.2		500.7		34.9	
1395	C5-1	6178.8	6288.40	42.9	46.43	1021.4	996.50	36	37.40
1396	C5-2	6146.1		53.2		1006.8		38.8	
1397	C5-3	6540.3		43.2		961.3		37.4	
1398	S1-1	664.7	647.93	200.5	198.40	1775.2	1806.93	25.1	24.47
1399	S1-2	632.9		198.7		1776		22.8	
1400	S1-3	646.2		196		1869.6		25.5	
1401	S2-1	555.1	556.93	150.1	151.00	1568.7	1606.37	25.5	24.37
1402	S2-2	542.5		153.4		1607.3		24.3	
1403	S2-3	573.2		149.5		1643.1		23.3	
1404	S3-1	751.6	720.60	38	35.60	2228.6	2207.53	31.8	31.87
1405	S3-2	703.9		33.6		2204		29.1	
1406	S3-3	706.3		35.2		2190		34.7	

Table III - 8

Raw data for 3050 extracts of 2 mm fraction. Concentrations are mg/l in solution and mg/kg soil.

Sample No.	Sample ID	As		Cr		Cu		Ni	
		mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg
1407	S4-1	3033.4	3157.73	37.5	43.20	3990.4	4228.03	35.4	35.27
1408	S4-2	3253.2		43.4		4425.1		36.1	
1409	S4-3	3186.6		48.7		4268.6		34.3	
1410	S5-1	2665.7	2711.77	43.7	48.50	3958.7	4009.17	32.1	33.97
1411	S5-2	2706.2		46.8		4002		33.2	
1412	S5-3	2763.4		55		4066.8		36.6	
1413	Blank	3.7		0.1		2.6		0	

Table III - 8

Sample No.	Sample ID	Pb		Ti		Zn	
		mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg
1383	C1-1	11764.3	11071.50	98.3	94.97	1649.8	1598.77
1384	C1-2	10436.8		91.4		1554.6	
1385	C1-3	11013.4		95.2		1591.9	
1386	C2-1	12034.4	12105.03	90.7	92.03	1651.5	1612.63
1387	C2-2	12385.9		95.4		1614.5	
1388	C2-3	11894.8		90		1571.9	
1389	C3-1	12139.4	10983.00	130.5	129.70	1677.7	1644.50
1390	C3-2	10265.3		127.9		1610.9	
1391	C3-3	10544.3		130.7		1644.9	
1392	C4-1	8592.7	8430.57	162.5	163.37	1670.4	1654.53
1393	C4-2	8235.1		167.5		1645	
1394	C4-3	8463.9		160.1		1648.2	
1395	C5-1	5879.2	5528.30	305.5	298.67	4896.6	4740.37
1396	C5-2	5234.6		301.2		4660.6	
1397	C5-3	5471.1		289.3		4663.9	
1398	S1-1	8636	8735.63	0	0.00	0	0.00
1399	S1-2	9002.6		0		0	
1400	S1-3	8568.3		0		0	
1401	S2-1	6721.6	6834.53	0	0.00	0	0.00
1402	S2-2	6912.1		0		0	
1403	S2-3	6869.9		0		0	
1404	S3-1	3655.9	3511.73	626.5	606.47	2593.8	2507.00
1405	S3-2	3413.3		590		2432.4	
1406	S3-3	3466		602.9		2494.8	

Table III - 8

Sample No.	Sample ID	Pb		Ti		Zn	
		mg/kg	Ave mg/kg	mg/kg	Ave mg/kg	mg/kg	Ave mg/kg
1407	S4-1	12090.2	12611.70	456.4	465.73	4062.3	4043.77
1408	S4-2	12944		478.6		4070.9	
1409	S4-3	12800.9		462.2		3998.1	
1410	S5-1	11414.1	11526.03	410	424.53	3317.8	3398.97
1411	S5-2	11538.3		419.4		3403.4	
1412	S5-3	11625.7		444.2		3475.7	
1413	Blank	0		0		0	

Table III - 9
Water Soluble Anions
1 g soil: 20 ml deionized water

				Cl				SO4-S			
Sample No.	Soil	Particle Size	Rep No.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.
1	C1	< 250 u	1	30.096	601.920	2545.073	2065.803	7730.82	154616.320	158281.387	3554.581
2	C1	< 250 u	2	235.74	4714.840			7925.69	158513.760		
3	C1	< 250 u	3	115.92	2318.460			8085.7	161714.080		
4	C2	< 250 u	1	41.327	826.540	2950.473	2321.336	4212.81	84256.220	83119.073	1022.727
5	C2	< 250 u	2	129.82	2596.360			4141.32	82826.440		
6	C2	< 250 u	3	271.43	5428.520			4113.73	82274.560		
7	C3	< 250 u	1	65.456	1309.120	1236.913	489.838	1151.3	23025.980	23368.440	296.777
8	C3	< 250 u	2	84.332	1686.640			1176.44	23528.820		
9	C3	< 250 u	3	35.749	714.980			1177.53	23550.520		
10	C4	< 250 u	1	42.398	847.960	1079.260	234.513	11278.1	225562.480	224252.567	2008.955
11	C4	< 250 u	2	53.648	1072.960			11262.8	225255.620		
12	C4	< 250 u	3	65.843	1316.860			11097	221939.600		
13	C5	< 250 u	1	93.71	1874.200	1710.733	209.521	10992.2	219843.500	219775.833	568.469
14	C5	< 250 u	2	89.173	1783.460			11015.4	220307.440		
15	C5	< 250 u	3	73.727	1474.540			10958.8	219176.560		
16	S1	< 250 u	1	48.607	972.140	943.580	251.459	65.792	1315.840	1307.840	222.088
17	S1	< 250 u	2	58.977	1179.540			76.291	1525.820		
18	S1	< 250 u	3	33.953	679.060			54.093	1081.860		
19	S2	< 250 u	1	60.716	1214.320	1171.887	166.808	161.003	3220.060	3286.960	80.668
20	S2	< 250 u	2	65.669	1313.380			163.214	3264.280		
21	S2	< 250 u	3	49.398	987.960			168.827	3376.540		
22	S3	< 250 u	1	78.519	1570.380	2198.007	761.098	523.075	10461.500	10509.247	322.839
23	S3	< 250 u	2	152.23	3044.580			510.647	10212.940		
24	S3	< 250 u	3	98.953	1979.060			542.665	10853.300		

Table III - 9
Water Soluble Anions
1 g soil: 20 ml deionized water

				Cl				SO4-S			
Sample No.	Soil	Particle Size	Rep No.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.
25	S4	< 250 u	1	47.34	946.800	976.267	59.943	144.122	2882.440	2898.127	84.825
26	S4	< 250 u	2	46.838	936.760			141.112	2822.240		
27	S4	< 250 u	3	52.262	1045.240			149.485	2989.700		
28	S5	< 250 u	1	55.385	1107.700	1129.060	109.770	127.221	2544.420	2528.933	22.359
29	S5	< 250 u	2	51.577	1031.540			126.954	2539.080		
30	S5	< 250 u	3	62.397	1247.940			125.165	2503.300		
31	E1	< 250 u	1	120.2	2404.060	4224.200	1735.229	10998.2	219963.560	221126.133	1046.384
32	E1	< 250 u	2	292.99	5859.760			11071.1	221422.400		
33	E1	< 250 u	3	220.44	4408.780			11099.6	221992.440		
34	E2	< 250 u	1	610.34	12206.800	9867.600	2355.434	17284.2	345684.600	347175.853	8516.450
35	E2	< 250 u	2	374.81	7496.260			16975.2	339503.520		
36	E2	< 250 u	3	494.99	9899.740			17817	356339.440		
37	E3	< 250 u	1	28.083	561.660	912.400	484.897	135.847	2716.940	2483.860	238.856
38	E3	< 250 u	2	35.49	709.800			111.981	2239.620		
39	E3	< 250 u	3	73.287	1465.740			124.751	2495.020		
40	E4	< 250 u	1	94.685	1893.700	2446.047	2318.228	67.099	1341.980	1359.853	21.299
41	E4	< 250 u	2	22.694	453.880			67.708	1354.160		
42	E4	< 250 u	3	249.53	4990.560			69.171	1383.420		
43	E5	< 250 u	1	762.39	15247.820	14171.107	991.351	470.357	9407.140	9449.087	104.160
44	E5	< 250 u	2	698.47	13969.340			468.622	9372.440		
45	E5	< 250 u	3	664.81	13296.160			478.384	9567.680		
46	C4	< 2mm	1	174.32	3486.380	3029.160	839.428	46.455	929.100	946.753	53.357
47	C4	< 2mm	2	177.04	3540.720			50.335	1006.700		
48	C4	< 2mm	3	103.02	2060.380			45.223	904.460		

Table III - 9
Water Soluble Anions
1 g soil: 20 ml deionized water

				Cl				SO4-S			
Sample No.	Soil	Particle Size	Rep No.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.	mg/L	mg/kg	Ave. mg/kg	Std. Dev.
49	S4	< 2mm	1	53.878	1077.560	10529.913	16603.182	11013.8	220275.160	225382.933	4921.286
50	S4	< 2mm	2	1485.1	29701.000			11504.7	230093.680		
51	S4	< 2mm	3	40.559	811.180			11289	225779.960		
52	BG	< 2mm	1	43.568	871.360	854.967	42.473	111.668	2233.360	1977.840	223.740
53	BG	< 2mm	2	44.34	886.800			94.156	1883.120		
54	BG	< 2mm	3	40.337	806.740			90.852	1817.040		

Table III - 9

Sample No.	Soil	Particle Size	Rep No.	NO3-N			
				mg/L	mg/kg	Ave. mg/kg	Std. Dev.
49	S4	< 2mm	1	12.7	254.340	290.880	37.929
50	S4	< 2mm	2	16.5	330.060		
51	S4	< 2mm	3	14.4	288.240		
52	BG	< 2mm	1	16.6	332.200	290.693	49.713
53	BG	< 2mm	2	11.8	235.600		
54	BG	< 2mm	3	15.2	304.280		

Table III - 9

Sample No.	Soil	Particle Size	Rep No.	NO3-N			
				mg/L	mg/kg	Ave. mg/kg	Std. Dev.
1	C1	< 250 u	1	6.7	134.060	102.547	46.097
2	C1	< 250 u	2	2.48	49.640		
3	C1	< 250 u	3	6.2	123.940		
4	C2	< 250 u	1	0	0.000	159.860	216.069
5	C2	< 250 u	2	3.7	73.900		
6	C2	< 250 u	3	20.3	405.680		
7	C3	< 250 u	1	13.8	276.840	100.313	153.351
8	C3	< 250 u	2	0	0.000		
9	C3	< 250 u	3	1.21	24.100		
10	C4	< 250 u	1	0	0.000	49.407	85.575
11	C4	< 250 u	2	0	0.000		
12	C4	< 250 u	3	7.41	148.220		
13	C5	< 250 u	1	9.24	184.700	297.260	216.399
14	C5	< 250 u	2	8.02	160.340		
15	C5	< 250 u	3	27.3	546.740		
16	S1	< 250 u	1	8.36	167.280	146.947	19.641
17	S1	< 250 u	2	7.27	145.480		
18	S1	< 250 u	3	6.4	128.080		
19	S2	< 250 u	1	43.6	871.020	941.693	83.249
20	S2	< 250 u	2	46	920.600		
21	S2	< 250 u	3	51.7	1033.460		
22	S3	< 250 u	1	22.2	443.500	507.000	60.980
23	S3	< 250 u	2	25.6	512.400		
24	S3	< 250 u	3	28.3	565.100		

Table III - 9

Sample No.	Soil	Particle Size	Rep No.	NO3-N			
				mg/L	mg/kg	Ave. mg/kg	Std. Dev.
25	S4	< 250 u	1	18.2	364.300	552.213	175.638
26	S4	< 250 u	2	29	580.100		
27	S4	< 250 u	3	35.6	712.240		
28	S5	< 250 u	1	41	820.580	628.227	166.631
29	S5	< 250 u	2	26.4	528.040		
30	S5	< 250 u	3	26.8	536.060		
31	E1	< 250 u	1	19.4	388.820	323.880	61.373
32	E1	< 250 u	2	15.8	315.980		
33	E1	< 250 u	3	13.3	266.840		
34	E2	< 250 u	1	124	2473.100	2471.267	147.079
35	E2	< 250 u	2	131	2617.420		
36	E2	< 250 u	3	116	2323.280		
37	E3	< 250 u	1	2.29	45.780	53.827	15.558
38	E3	< 250 u	2	3.59	71.760		
39	E3	< 250 u	3	2.2	43.940		
40	E4	< 250 u	1	4.98	99.580	77.520	69.180
41	E4	< 250 u	2	0	0.000		
42	E4	< 250 u	3	6.65	132.980		
43	E5	< 250 u	1	44.3	885.540	875.880	9.226
44	E5	< 250 u	2	43.7	874.940		
45	E5	< 250 u	3	43.4	867.160		
46	C4	< 2mm	1	43.8	875.500	753.220	130.242
47	C4	< 2mm	2	38.4	767.900		
48	C4	< 2mm	3	30.8	616.260		

Table III - 9

Sample No.	Soil	Particle Size	Rep No.	NO3-N			
				mg/L	mg/kg	Ave. mg/kg	Std. Dev.
49	S4	< 2mm	1	12.7	254.340	290.880	37.929
50	S4	< 2mm	2	16.5	330.060		
51	S4	< 2mm	3	14.4	288.240		
52	BG	< 2mm	1	16.6	332.200	290.693	49.713
53	BG	< 2mm	2	11.8	235.600		
54	BG	< 2mm	3	15.2	304.280		

Table III - 10
Toxicity Characteristic Leaching Procedure (TCLP)
1 g:20 ml, shake rt 18 hrs

Sample No.	Soil	Particle Size	Rep No.	As mg/L		As mg/kg	Average As, mg/kg	Standard Deviation	Bioavailable As, %
2853	C1	< 250 u	1	3.028	2.969	60.56	59.38	1.03	0.53
2854	C1	< 250 u	2	2.934		58.68			
2855	C1	< 250 u	3	2.945		58.90			
2856	C2	< 250 u	1	3.095	3.0137	61.90	60.27	1.68	0.35
2857	C2	< 250 u	2	3.019		60.38			
2858	C2	< 250 u	3	2.927		58.54			
2859	C3	< 250 u	1	3.035	3.0267	60.70	60.53	0.16	0.45
2860	C3	< 250 u	2	3.019		60.38			
2861	C3	< 250 u	3	3.026		60.52			
2862	C4	< 250 u	1	2.98	2.941	59.60	58.82	0.68	0.51
2863	C4	< 250 u	2	2.924		58.48			
2864	C4	< 250 u	3	2.919		58.38			
2865	C5	< 250 u	1	2.957	3.0007	59.14	60.01	0.77	0.96
2866	C5	< 250 u	2	3.016		60.32			
2867	C5	< 250 u	3	3.029		60.58			
2868	S1	< 250 u	1	3.082	2.961	61.64	59.22	2.28	14.62
2869	S1	< 250 u	2	2.945		58.90			
2870	S1	< 250 u	3	2.856		57.12			
2871	S2	< 250 u	1	2.788	2.7817	55.76	55.63	2.49	12.36
2872	S2	< 250 u	2	2.903		58.06			
2873	S2	< 250 u	3	2.654		53.08			
2874	S3	< 250 u	1	2.803	2.9057	56.06	58.11	1.79	4.92
2875	S3	< 250 u	2	2.965		59.30			
2876	S3	< 250 u	3	2.949		58.98			
2877	S4	< 250 u	1	3.108	3.172	62.16	63.44	1.16	1.26
2878	S4	< 250 u	2	3.221		64.42			
2879	S4	< 250 u	3	3.187		63.74			
2880	S5	< 250 u	1	2.95	2.9293	59.00	58.59	0.82	1.26
2881	S5	< 250 u	2	2.882		57.64			
2882	S5	< 250 u	3	2.956		59.12			
2883	E1	< 250 u	1	2.643	2.649	52.86	52.98	0.55	16.01
2884	E1	< 250 u	2	2.679		53.58			
2885	E1	< 250 u	3	2.625		52.50			
2886	E2	< 250 u	1	2.792	2.754	55.84	55.08	0.67	23.64
2887	E2	< 250 u	2	2.741		54.82			
2888	E2	< 250 u	3	2.729		54.58			

Table III - 10
Toxicity Characteristic Leaching Procedure (TCLP)
1 g:20 ml, shake rt 18 hrs

Sample No.	Soil	Particle Size	Rep No.	As mg/L		As mg/kg	Average As, mg/kg	Standard Deviation	Bioavailable As, %
2889	E3	< 250 u	1	2.579	2.6117	51.58	52.23	0.57	6.53
2890	E3	< 250 u	2	2.629		52.58			
2891	E3	< 250 u	3	2.627		52.54			
2892	E4	< 250 u	1	2.474	2.725	49.48	54.50	4.35	3.73
2893	E4	< 250 u	2	2.86		57.20			
2894	E4	< 250 u	3	2.841		56.82			
2895	E5	< 250 u	1	3.021	2.9493	60.42	58.99	1.31	14.71
2896	E5	< 250 u	2	2.934		58.68			
2897	E5	< 250 u	3	2.893		57.86			
2898	C4	< 2mm	1	2.707	2.8317	54.14	56.63	1.64	
2899	C4	< 2mm	2	2.836		56.72			
2900	C4	< 2mm	3	2.952		59.04			
2901	S4	< 2mm	1	3.5	3.418	70.00	68.36	3.71	
2902	S4	< 2mm	2	3.508		70.16			
2903	S4	< 2mm	3	3.246		64.92			
2904	BG	< 2mm	1	2.767	2.7577	55.34	55.15	0.06	
2905	BG	< 2mm	2	2.751		55.02			
2906	BG	< 2mm	3	2.755		55.10			

Table III - 11
Soil Texture - Pipette Method

Sample ID	Very Coarse Sand 1.0-2.0 mm	Coarse Sand 0.50-1.0 mm	Medium Sand 0.25-0.50 mm	Fine Sand .10-0.25 m	Very Fine Sand 0.05-0.10 mm	Coarse Silt .02-0.05 m	Medium Silt .005-0.02 m	Fine Silt .002-0.005 m	Clay 0.002 m
C1	0.1	0.9	1.1	22.8	23.5	20.8	18.1	7.0	5.7
C2	0.0	0.1	0.2	28.5	21.3	20.8	14.7	4.2	10.1
C3	0.0	0.0	0.0	19.2	23.5	22.9	16.6	4.5	13.4
C4	0.0	0.0	0.6	29.2	25.0	22.2	10.9	2.6	9.8
C5	0.0	0.0	0.1	20.5	30.3	24.7	13.7	3.6	7.4
S1	0.0	0.0	0.1	49.7	19.8	14.6	7.5	1.6	6.5
S2	0.0	0.0	0.0	20.7	20.1	20.9	13.5	6.3	18.4
S3	0.0	0.0	0.0	39.6	23.4	16.6	8.9	2.6	8.4
S4	0.0	0.0	0.1	33.3	21.3	22.1	12.2	3.5	7.3
S5	0.0	0.0	0.0	34.0	20.3	21.5	12.9	3.7	7.5
BG	1.9	6.4	6.9	15.5	14.2	24.6	13.4	5.0	12.2

Sample ID	Texture	< 50 um
C1	Silt Loam	51.6
C2	Loam	49.8
C3	Loam	57.4
C4	Silt Loam	45.5
C5	Loam	49.4
S1	Fine Silt Loam	30.2
S2	Loam	59.1
S3	Fine Silt Loam	36.5
S4	Fine Silt Loam	45.1
S5	Fine Silt Loam	45.6
BG	Loam	55.2

Note: All soils above, except BG, are the 250 micron fraction. The BG sample is < 2

Appendix IV

Biomethylation Data

Table IV - 1

Biomethylation Data

	Arsenic, micrograms									
	Week 2					Week 4				
	KI-1	KI-2	Total	AC	As	KI-1	KI-2	Total	AC	As
Treatment-Rep	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total
OCB1	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.033	0.033
OCB2	0.000	0.000	0.000	0.060	0.060	0.000	0.000	0.000	3.442	3.442
OCB3	0.000	0.000	0.000	0.020	0.020	0.000	0.000	0.000	0.317	0.317
OCB4	0.000	0.000	0.000	6.599	6.599	0.000	0.000	0.000	0.377	0.377
OCN1	0.000	0.000	0.000	0.074	0.074	0.000	0.000	0.000	0.094	0.094
OCN2	0.000	0.000	0.000	0.328	0.328	0.000	0.000	0.000	0.170	0.170
OSB1	0.000	0.000	0.000	**105.316**	105.316	0.025	0.000	0.025	0.051	0.076
OSB2	0.000	0.000	0.000	0.093	0.093	0.191	0.081	0.272	0.092	0.364
OSB3	0.000	0.000	0.000	0.068	0.068	0.022	0.000	0.022	0.048	0.070
OSB4	0.000	0.000	0.000	0.031	0.031	0.060	0.000	0.060	0.080	0.140
OSN1	0.000	0.000	0.000	0.289	0.289	0.022	0.000	0.022	0.000	0.022
OSN2	0.000	0.000	0.000	**7.557**	7.557	0.000	0.000	0.000	0.000	0.000
ACB1	0.000	0.000	0.000	0.082	0.082	0.000	0.000	0.000	0.000	0.000
ACB2	0.000	0.000	0.000	0.104	0.104	0.000	0.027	0.027	0.101	0.128
ACB3	0.000	0.000	0.000	0.042	0.042	0.024	0.000	0.024	0.000	0.024
ACB4	0.000	0.000	0.000	0.113	0.113	0.000	0.000	0.000	0.064	0.064
ACN1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.248	0.248
ACN2	0.000	0.000	0.000	0.096	0.096	0.000	0.015	0.015	0.031	0.046
ASB1	0.000	0.000	0.000	0.164	0.164	0.000	0.038	0.038	0.039	0.077
ASB2	0.000	0.000	0.000	0.278	0.278	0.029	0.000	0.029	0.231	0.260
ASB3	0.000	0.000	0.000	0.120	0.120	0.091	0.060	0.151	0.385	0.536
ASB4	0.000	0.000	0.000	0.155	0.155	0.036	0.000	0.036	0.241	0.277
ASN1	0.000	0.000	0.000	**5.773**	5.773	0.129	0.036	0.165	0.027	0.192
ASN2	0.000	0.000	0.000		0.000	0.191	0.045	0.236	0.273	0.509

The TMA numbers in bold (OSB1, OSN2, and ASN1) are clearly outliers. These values will be omitted on the following sheet

Table IV - 1
Biomethylation Data

	Week 8					Week 12				
	KI-1	KI-2	Total	AC	As	KI-1	KI-2	Total	AC	As
Treatment-Rep	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total
OCB1	0.044	0.027	0.071	0.068	0.139	0.112	0.010	0.122	0.097	0.219
OCB2	0.027	0.036	0.063	0.041	0.104	0.056	0.004	0.060	0.086	0.146
OCB3	0.023	0.030	0.053	0.040	0.093	2.978	0.079	3.057	0.194	3.251
OCB4	0.017	0.016	0.033	0.041	0.074	0.390	0.137	0.527	0.075	0.602
OCN1	0.019	0.024	0.043	0.025	0.068	0.081	0.000	0.081	0.057	0.138
OCN2	0.014	0.020	0.034	0.029	0.063	0.196	0.000	0.196	0.143	0.339
OSB1	0.029	0.010	0.039	0.044	0.083	0.000	0.000	0.000	0.080	0.080
OSB2	0.016	0.007	0.023	0.251	0.274	0.000	0.000	0.000	0.029	0.029
OSB3	0.016	0.023	0.039	0.041	0.080	0.000	0.330	0.330	0.299	0.629
OSB4	0.031		0.031	0.079	0.110	0.021	0.014	0.035	0.030	0.065
OSN1	0.010	0.012	0.022	0.285	0.307	0.021	0.016	0.037	0.046	0.083
OSN2	0.018	0.012	0.030	0.092	0.122	0.016	0.040	0.056	0.106	0.162
ACB1	0.013	0.009	0.022	0.072	0.094	0.015	0.018	0.033	0.026	0.059
ACB2	0.029	0.016	0.045	0.050	0.095	0.033	0.010	0.043	0.011	0.054
ACB3	0.001	0.000	0.001	0.059	0.060	0.000	0.000	0.000	0.020	0.020
ACB4	0.000	0.000	0.000	0.061	0.061	0.005	0.000	0.005	0.033	0.038
ACN1	0.000	0.018	0.018	0.050	0.068	0.000	0.000	0.000	0.041	0.041
ACN2	0.031	0.036	0.067	0.058	0.125	0.000	0.000	0.000	0.211	0.211
ASB1	0.038	0.010	0.048	0.054	0.102	0.004	0.000	0.004	0.33	0.334
ASB2	0.038	0.048	0.086	0.343	0.429	0.038	0.020	0.058	0.246	0.304
ASB3	0.059	0.047	0.106	0.118	0.224	0.016	0.000	0.016		0.016
ASB4	0.034	0.035	0.069	0.118	0.187	0.008	0.000	0.008		0.008
ASN1	0.049	0.036	0.085	0.176	0.261	0.050	0.001	0.051	0.215	0.266
ASN2	0.124	0.059	0.183	0.283	0.466	0.000	0.000	0.000	0.051	0.051

Table IV - 1
Biomethylation Data

	Week 16					Week 20				
	KI-1	KI-2	Total	AC	As	KI-1	KI-2	Total	AC	As
Treatment-Rep	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total	MMA+DMA	MMA+DMA	MMA+DMA	TMA	Total
OCB1	0.014	0.057	0.071	0.253	0.324	0.038535	0.003295	0.04183	0.09785	0.13968
OCB2	1.000	0.075	1.075	0.291	1.366	0.03093	0	0.03093	0.00221	0.03314
OCB3	2.412	0.059	2.471	0.200	2.671	0.04867	0	0.04867	0	0.04867
OCB4	0.673	0.143	0.816	0.174	0.990	0.13375	0	0.13375	0.05215	0.1859
OCN1	0.148	0.069	0.217	0.315	0.532	0	0.007615	0.007615	0.00105	0.00867
OCN2	0.115	0.057	0.172	0.128	0.300	0.025595	0.007985	0.03358	0.01213	0.04571
OSB1	0.069	0.043	0.112	0.101	0.213	0.07785	0.08525	0.1631	0	0.1631
OSB2	0.062	0.065	0.127	0.118	0.245	0.002445	0.001715	0.00416	0.4783	0.48246
OSB3	0.120	0.055	0.175	0.141	0.316	0.037545	0.07085	0.108395	0.01508	0.12348
OSB4	0.071	0.030	0.101	0.120	0.221	0.02483	0.026245	0.051075	0	0.05108
OSN1	0.039	0.023	0.062	0.122	0.184	0.1334	0.07085	0.20425	0.10245	0.3067
OSN2	0.040	0.060	0.100	0.105	0.205	0.04221	0	0.04221	0.00716	0.04937
ACB1	0.036	0.012	0.048	0.066	0.114	0.001455	0	0.001455	0.0078	0.00925
ACB2	0.026	0.041	0.067	0.150	0.217	0	0.00974	0.00974	0.0163	0.02604
ACB3	0.039	0.185	0.224	0.109	0.333	0	0.028635	0.028635	0	0.02864
ACB4	0.109	0.105	0.214	0.116	0.330	0	0.003	0.002515	0.2045	0.20702
ACN1	0.076	0.063	0.139	0.113	0.252	0.000	0.037	0.03677	0	0.03677
ACN2	0.062	0.045	0.107	0.108	0.215	0.000	0.000	0.00003	0.0421	0.04213
ASB1	0.130	0.074	0.204	0.152	0.356	0	0.000385	0.000385	0.01189	0.01227
ASB2	0.081	0.063	0.144	0.115	0.259	0.006375	0.013315	0.01969	0.43695	0.45664
ASB3	0.069	0.066	0.135	0.053	0.188	0.03234	0.01321	0.04555	0.2894	0.33495
ASB4	0.070	0.077	0.147	0.085	0.232	0.00370500	0.01047000	0.014175	0.4639	0.47808
ASN1	0.065	0.060	0.125	0.071	0.196	0.00911500	0.00616000	0.015275	0.1476	0.16288
ASN2	0.085	0.062	0.147	0.042	0.189	0.01414000	0.00947500	0.023615	0.1662	0.18982

Table IV - 2
Biomethylated arsenic - lead stabilized calcine experiment

	Week 2		Week 2		Week 8		Week 12	
Sample ID	As ug/L	Total As ug As	As ug/L	Total As ug As	As ug/L	Total As ug As	As ug/L	Total As ug As
LS1-KI	3.579	0.119199	11.448	0.5724	27.823	2.782	296.025	29.603
LS2-KI	2.74	0.137	1.011	0.05055	9.27	0.927	17.674	1.767
LS3-KI	3.189	0.15945	1.693	0.08465	663.397	66.340	1263.715	126.372
LS1-AC	1.292	0.065	0	0.000	0	0.000	166.126	8.306
LS2-AC	4.273	0.214	4.768	0.238	9.069	0.453	3.981	0.199
LS3-AC	1.901	0.095	10.226	0.511	0	0.000	31.282	1.564

	Week 16		Week 20	
Sample ID	As ug/L	Total As ug As	As ug/L	Total As ug As
LS1-KI	249.1	24.909	21.743	2.174
LS2-KI	6.639	0.664	94.306	9.431
LS3-KI	102.1	10.211	7.932	0.793
LS1-AC	14.5	0.725	4.301	0.215
LS2-AC	1.161	0.058	4.743	0.237
LS3-AC	4.685	0.234	1.885	0.094

Appendix V

Quality Assurance Work Plan

QUALITY ASSURANCE

Intended Use of the Data and Acceptable Criteria for Data Quality

Collected data will be used to evaluate the ability of proposed chemical and *in-vitro* methods to provide a better, site-specific determination of the bioavailability of metals in soils/solid wastes. A more reasonable, site-specific method of quantifying the bioavailable fractions of metals will provide a more reasonable estimation of the true risk from exposure to contaminated environmental media by lowering the uncertainty associated with the fraction of As absorbed in risk calculations. All reagents and solutions utilized for the proposed study will be of high purity Optima™ quality to ensure negligible As contamination. Criteria used to determine the acceptability of data include the evaluation of: 1) precision, by determining the degree of reproducibility of data as from the analysis of sample replications in the laboratory and replications of swine to be exposed to each soil treatment; 2) accuracy, by determining spike recoveries in analytical samples and analyzing appropriate reference standards and blanks; 3) representativeness, in a qualitative way, by collecting contaminated materials representative of metals contaminated sites and choosing representative swine for feeding trials; 4) completeness, by providing statistically sound experimental designs and appropriate replications to ensure $p<0.05$; and 5) comparability, by performing statistical correlations of the *in-vivo* data with the chemical and *in-vitro* data.

Project Requirements for Precision, Accuracy, Representativeness, Completeness, and Comparability (PARCS)

Acceptability of data will be determined by following procedures described in USEPA (1988). The specific PARCS parameters will be evaluated as: 1) Precision, by analyzing duplicate samples and performing replicates of experimental procedures. Laboratory duplicates will be analyzed at the rate of 10% of the samples. The relative percent difference shall not be greater than 5%. 2) Accuracy, by analysis of spiked analytical samples and appropriate reference materials. Spike recoveries shall be between 80 and 120% and reference materials shall be within 5% of the reported value. 3) Representativeness, will be evaluated qualitatively. 4) Completeness will be accomplished by ensuring all samples are analyzed and data collected. 5) Comparability will be evaluated statistically by Analysis of Variance (ANOVA) techniques with statistical significance level reported. Correlations between *in-vivo*, chemical, and *in-vitro* methods results will be determined.

Procedures for Collection and Preparation of Samples

Solid waste/soil materials have been collected from a mining, milling, and smelter site. Remedial Investigation reports and an As-specific field test kit were used to estimate the total As prior to collection. Samples were collected using decontaminated collection equipment. All samples were transported and stored under proper chain-of-custody procedures to insure maintenance of sample integrity. Soil sample preparation, including air drying, sieving, and homogenizing, will be performed by Oklahoma State University. Prepared soil materials will be sent to University of Missouri-Columbia under proper chain-of-custody procedures. Urine samples will be collected, filtered, acidified by University of

Missouri-Columbia then shipped to Oklahoma State University packed in ice (cool, 4° C). Tissue samples will be collected, prepared by University of Missouri-Columbia and then shipped to Oklahoma State University packed in dry ice.

Procedures for Sample Handling, Identification, Preservation, Transportation, and Storage

Soil/solid waste materials will be stored in air-tight containers in a locked soil laboratory in which only authorized personnel have access. A lettering and numerical system will be utilized to identify all soil samples, *e.g.*, slag materials will be labeled with "S" and calcine materials will be labeled "C". Roman numerals will then be used to represent the As level, *e.g.*, "I" will be used to represent the lowest level of As, "II" will represent the next highest level, and so forth. Numbers will be used, such as -1, 1, 3, 6, 9, 12, and 15, to designate on which day of the exposure the swine urine sample was collected. Replicate numbers will be represented as the last number in the identification label. As an example, the second replication of a swine urine sample collected on day 3 following exposure to the lowest level of soil As would be represented as follows: "CI33". All samples will be labeled with black permanent markers directly on the container. Biological samples will be collected and prepared by UMC and transported to OSU under chain-of-custody procedures. Urine samples will be stored in a dedicated refrigerator located in the Chemistry Laboratory for Environmental Analysis at OSU which is kept locked and only accessed by authorized personnel. All sample transport will be accomplished via an overnight express delivery company.

Analytical Methods and Test Procedures

Standardized analytical methods will be performed per USEPA SW-846 Method 7062 for hydride generation and subsequent determination of As in soil extracts, in-vitro digests, and animal urine samples. Other test procedures to characterize As in contaminated materials will include total metals (USEPA SW-846 Method 3050) and Toxicity Characteristic Leaching Procedure (USEPA SW-846 Method 1311).

Standard QA/QC Control Procedures

General Statement of Intent to Comply with GLP (40CFR Ch. 1 Part 792; 7-1-90): It is the intent of the Principal Investigator, Co-Investigator, and all associated analytical personnel that this applied research investigation comply with good Laboratory Practice Procedures (GLP) as outlined in 40CFR, Chapter 1. We will follow a Quality Assurance Project Plan (QAPP) and the associated Standard Operating Procedures (SOPs) developed according to USEPA guidance as appropriate for any work to obtain data from CERCLA sites (USEPA, 1988a, 1988b, and 1993). Where quality control and assurances do not strictly follow GLP as outlined in 40CFR, Chapter 1, the spirit of GLP will be followed in such a manner as to provide equivalent or comparable quality control and assessment to assure a defensible, scientific process.

VITA

Robin R. Rodriguez

Candidate for the Degree of

Doctor of Philosophy

Thesis: BIOAVAILABILITY AND BIOMETHYLATION OF ARSENIC IN CONTAMINATED SOILS AND SOLID WASTES

Major field: Soil Science

Minor Field: Environmental Engineering

Biographical:

Education: Graduated from Pattonville Senior High School, Maryland Heights, Missouri in May 1975; received Bachelor of Science degree and Master of Science degree in Soil Science from Utah State University, Logan, Utah, in May of 1979 and 1981, respectively; received a Master of Science degree in Civil Engineering from the University of Missouri, Columbia, Missouri, in May 1994. Completed the requirements for the Doctor of Philosophy degree with a major in Soil Science and minor in Environmental Engineering at Oklahoma State University, Stillwater, Oklahoma in May 1998.

Experience: Worked as Supervisor-Soil Testing Laboratory, University of Missouri, Columbia, Missouri from 1982 until 1991. Employed by Sverdrup Environmental, Inc., Maryland Heights, Missouri, as a consulting engineer (hazardous waste) from 1991 to 1994.

Professional Memberships: Society of Environmental Toxicology and Chemistry.